Vol. XXVIII. *June, 1915.* *No. 6.*

BIOLOGICAL BULLETIN

THE MARINE BIOLOGICAL LABORATORY

SEVENTEENTH REPORT

FOR THE YEAR 1914

I. TRUSTEES

EX OFFICIO

FRANK R. LILLIE, *Director*, The University of Chicago.
GILMAN A. DREW, *Assistant Director*, Marine Biological Laboratory.
D. BLAKELY HOAR, *Treasurer*, 161 Devonshire Street, Boston, Mass.
GARY N. CALKINS, *Clerk of the Corporation*, Columbia University.

TO SERVE UNTIL 1918

CORNELIA M. CLAPP.....Mount Holyoke College.
E. G. CONKLIN.....Princeton University.
ROSS G. HARRISON.....Yale University.
CAMILLUS G. KIDDER.....27 William Street, New York City.
M. M. METCALF.....Oberlin College.
WILLIAM PATTEN.....Dartmouth College.
JACOB REIGHARD.....University of Michigan.
W. B. SCOTT.....Princeton University.

TO SERVE UNTIL 1917

S. F. CLARKE.....Williams College.
CHARLES A. COOLIDGE.....Ames Building, Boston, Mass.
C. R. CRANE.....Woods Hole, Mass., President of the Board.
ALFRED G. MAYER.....Carnegie Institution.
C. E. MCCLUNG.....University of Pennsylvania.
T. H. MORGAN.....Columbia University.

ERWIN F. SMITH.....United States Department of Agriculture.
E. B. WILSON.....Columbia University.

TO SERVE UNTIL 1916

H. H. DONALDSON.....Wistar Institute of Anatomy and Biology.
M. J. GREENMAN.....Wistar Institute of Anatomy and Biology.
C. W. HARGITT.....Syracuse University.
H. S. JENNINGS.....Johns Hopkins University.
GEORGE LEFEVRE.....University of Missouri, Secretary of the Board.
A. P. MATHEWS.....University of Chicago.
G. H. PARKER.....Harvard University.
HENRY B. WARD.....University of Illinois.

TO SERVE UNTIL 1915

H. C. BUMPUS.....Tufts College.
R. A. HARPER.....Columbia University.
W. A. LOCY.....Northwestern University.
JACQUES LOEB.....The Rockefeller Institute for Medical Research.
F. P. MALL.....Johns Hopkins University.
GEORGE T. MOORE.....Missouri Botanical Garden, St. Louis.
L. L. NUNN.....Telluride, Colo.
JOHN C. PHILLIPS.....299 Berkeley Street, Boston, Mass.

II. ACT OF INCORPORATION

No. 3170.

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have com-

plied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our LORD ONE THOUSAND, EIGHT HUNDRED and EIGHTY-EIGHT.

HENRY B. PIERCE,
Secretary of the Commonwealth.

[SEAL.]

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The annual meeting of the members shall be held on the second Tuesday in August, at the Laboratory, in Woods Hole, Mass., at 12 o'clock noon, in each year, and at such meeting the members shall choose by ballot a Treasurer and a Clerk, who shall be, *ex officio*, members of the Board of Trustees, and Trustees as hereinafter provided. At the annual meeting to be held in 1897, not more than twenty-four Trustees shall be chosen, who shall be divided into four classes, to serve one, two, three, and four years, respectively, and thereafter not more than eight Trustees shall be chosen annually for the term of four years. These officers shall hold their respective offices until others are chosen and qualified in their stead. The Director and Assistant Director, who shall be chosen by the Trustees, shall also be Trustees, *ex officio*.

II. Special meetings of the members may be called by the Trustees, to be held in Boston or in Woods Hole at such time and place as may be designated.

III. The Clerk shall give notice of meetings of the members by

publication in some daily newspaper published in Boston at least fifteen days before such meeting, and in case of a special meeting the notice shall state the purpose for which it is called.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. The Trustees shall have the control and management of the affairs of the Corporation; they shall present a report of its condition at every annual meeting; they shall elect one of their number President and may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

VI. Meetings of the Trustees shall be called by the President, or by any two Trustees, and the Secretary shall give notice thereof by written or printed notice sent to each Trustee by mail, postpaid. Seven Trustees shall constitute a quorum for the transaction of business. The Board of Trustees shall have power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient.

VII. The President shall annually appoint two Trustees, who shall constitute a committee on finance, to examine from time to time the books and accounts of the Treasurer, and to audit his accounts at the close of the year. No investments of the funds of the Corporation shall be made by the Treasurer except approved by the finance committee in writing.

VIII. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be given to the Boston Society of Natural History, or some similar public institution, on such terms as may then be agreed upon.

IX. These By-Laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-Laws will be acted upon.

X. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

IV. TREASURER'S REPORT

Cash Receipts and Disbursements For the Year Ending December 31, 1914

Receipts

Cash on hand January 1, 1914	$ 4,663.05	
Annual dues	1,024.00	
Biological Bulletin	1,221.12	
Dexter House	4,151.18	
Charles R. Crane	34,200.00	
Donations	35.14	
Dormitory, stone building	424.60	
Dormitory, Whitman Cottage	252.75	
Fish Trap	995.24	
Instruction, botany	750.00	
Instruction, embryology	1,000.00	
Instruction, physiology	500.00	
Instruction, zoology	2,100.00	
Mess	12,749.22	
Miscellaneous	620.15	
Research	2,950.00	
Supply department	14,003.35	$81,639.80

Payments

Administration	$ 7,202.56	
Biological Bulletin	1,771.64	
Boats	5,604.42	
Carpenter shop	288.27	
Chemical department	2,640.20	
Dexter House	4,104.87	
Dormitory, stone building	204.06	
Dormitory, Whitman House	148.37	
Dredge	1,751.00	
Fish Trap	1,206.41	
Instruction, botany	950.00	
Instruction, embryology	475.00	
Instruction, physiology	650.00	

Instruction, zoölogy	1,139.00	
Lectures	18.85	
Library	3,000.88	
Maintenance of buildings and grounds	4,573.61	
Mess	17,665.68	
Miscellaneous	1,569.59	
New laboratory (furniture and equipment)	10,263.10	
Philosophical lectures	100.00	
Pumping station	3,892.38	
Supply department	10,295.75	
Supply department improvements	437.48	
Cash on hand January 1, 1915	1,686.68	$81,639.80

CASH RECEIPTS AND DISBURSEMENTS ON ACCOUNT OF FUNDS, AUGUST 1, 1914 TO JANUARY 1, 1915

RESERVE FUND

Cash on hand August 1, 1914	$107.77	
Div. 14 shs. United Shoe Mach. Corp. Pfd.	5.25	
Div. 6 shs. Am. Smelting & Refining Co. Pfd.	21.00	
Div. 8 shs. Gen. Elec. Co.	16.00	
Interest on deposit	.28	$150.30

LUCRETIA CROCKER FUND

Cash on hand August 1, 1914	$136.29	
Div. 1 sh. West End St. Ry. Co.	1.75	
Div. 18 shs. Vermont & Mass. Ry. Co.	54.00	
Div. 2½ shs. Gen. Elec. Co.	5.00	
Div. 1 sh. Am. Tel. & Tel. Co.	2.00	
	$199.04	
1914 scholarship	100.00	99.04

LIBRARY FUND

Cash on hand August 1, 1914	$157.79	
Div. 5 shs. United Shoe Mach. Corp. Pfd.	1.87	
Div. 1 sh. Am. Smelting & Refining Co. Pfd.	3.50	
Div. 3 shs. Am. Tel. & Tel. Co.	6.00	
Div. 2½ shs. Gen. Elec. Co.	5.00	174.16
Cash on hand January 1, 1915		$423.50

HARVEY S. CHASE AND COMPANY
Certified Public Accountants
84 State Street
Boston

December 18, 1914.

D. BLAKELY HOAR, ESQ.,
Treasurer, Marine Biological
Laboratory at Woods Hole, Mass.,
161 Devonshire Street, Boston.

Dear Sir: We have audited the accounts of the Marine Biological Laboratory as kept at Woods Hole, and of the Trust Funds and accounts as kept at your office, 161 Devonshire Street, for the year ended December 31, 1913.

We have checked the report of the Treasurer, submitted to us, and find it correct and in accordance with the books and accounts of the Laboratory. The extent of the audit is set forth in a detailed report under date of November 25, 1914.

Very respectfully,
HARVEY S. CHASE & CO.

COST OF THE NEW LABORATORY, BY CONTRACT, PAID DIRECTLY FROM MR. CRANE'S OFFICE

L. D. Willcut Sons Co., Builders	$77,810.32
C. E. Cronin, Plumbing	9,482.52
Edwin Lewis, Electrician	3,479.00
Cleghorn Heating Co., Heating	3,132.55
Otis Elevator Co., Elevator	1,485.00
Simpson Bros., Sinks	360.00
Monroe Refrigerator Co.	195.00
Architect's Commission	4,842.61
	$100,787.00

V. LIBRARIAN'S REPORT

AUGUST, 1914

All who have worked in Woods Hole for any length of time must appreciate how grateful we are to have the library so well cared for, now, in the new fireproof building. It is certainly a great relief to fear no longer its sudden destruction. In addition

to this, the librarian may here announce the appointment of an assistant librarian to be in charge at the Laboratory throughout the year. This has been a pressing need for several years. It assures continuity of the work and has made possible many important improvements. We were fortunate to secure for this position Miss May E. Scott, who started work in April, after some weeks devoted to special training. In the meantime Dr. Drew supervised the moving and installation of the books in the new library.

The improvements noted below have been accomplished through Miss Scott's skillful management and very great industry. Unfortunately she has been ill since the middle of June, but her work was already so well in hand that it has been possible to continue most of it during this season. We must thank Miss Elizabeth Dunn for her valuable services in this emergency during the summer. Without her generous efforts in behalf of Miss Scott and the library we should have been seriously embarrassed. In addition to this we have been much helped by having extra assistance for two hours a day for the heavy summer routine.

The entire library has been re-accessioned, and new catalogs made. There are over 3,300 volumes exclusive of the reprints, which number over 1,500. A modern system has been introduced throughout, which will make the library much more accessible next year. Many missing parts were secured; and more than 500 volumes were bound, necessitating an extra expenditure of $500. Several notable additions have been made to the library:

The American Museum of Natural History has loaned us indefinitely a number of their duplicate sets and books which will be of great assistance. These works are not yet catalogued, but there will be over 2,500 volumes, among which is a number of useful sets of journals, and of memoirs, transactions, and proceedings, of academies, etc., hitherto not on our shelves.

The Journal of Biological Chemistry was added, the back volumes being a gift from the editors.

Dr. Beyer presented several boxes of books containing among other things a duplicate set of the *American Journal of Physiology*,

and a set of the *Journal of Medical Research;* also *Zeitschrift für Hygiene*, and the *Archiv für Schiffs- und Tropenhygiene.*

Dr. Kellicott gave, in addition to his own text-books, about 100 volumes.

Dr. Otto Glaser sent a set of the *Proceedings of the Society of Experimental Biology and Medicine*, with other volumes.

Dr. Duggar has obtained a lot of much needed pamphlets missing from sets.

Mrs. Edward Gardiner added a number of volumes to her former gift.

Drs. Loeb, Minot, Hegner, McFarland, and Mrs. Agassiz and Mr. Crane presented books recently published.

The book-publishing companies have continued to present new books, Blakiston forwarding this week 14 volumes on chemistry, etc.

Stechert has inaugurated a new plan of sending books recently issued for examination. This was done at Dr. Oliver Strong's request, and has proved to be a great convenience.

Dr. H. F. Osborn gave a volume, and has turned over to us the premium on sales of one of his books. This will be continued.

Dr. E. E. Just has offered $5.00 for five years to assist us in securing more journals. Dr. Rice has promised $10 per year for the same purpose; Dr. E. B. Meigs gave $10.00 also, and the amount already on hand, announced last year for this fund, now totals $50.00, which will be expended for certain missing volumes of our files. We have acknowledged a number of reprints and books, but feel that here is a place where biologists may help us still more. A word to others and to publishers may greatly aid us.

The library has now reached a stage of stability. It is well housed, well cared for, of great use, and rapidly growing. We should be spending at least twice the present amount (that is about $1,000 a year more for books); especially for a number of journals, now much in demand. There should be a few more works of general character. Members of the corporation might greatly extend the library with a little effort. The exchange list might be further extended as soon as conditions become quiet abroad.

There is little to be added to the material equipment; beyond a truck with shelves for shifting volumes, and some suitable arrangement, possibly glass gallery platforms, to make the upper shelves readily accessible.

H. McE. KNOWER,
Librarian.

Aug. 11, 1914.

VI. THE DIRECTOR'S REPORT

TO THE TRUSTEES OF THE MARINE BIOLOGICAL LABORATORY:

Gentlemen: I have the honor to transmit herewith a report of the twenty-seventh session of the Marine Biological Laboratory, for the year 1914. The number of investigators in attendance was 128, as compared with 122 in 1913, 93 in 1912, and 82 in 1911. The number of students in the courses was 89 as compared with 69 in 1913, 67 in 1912, and 65 in 1911. The total attendance was 217 as compared with 191 in 1913, 160 in 1912, and 147 in 1911. The number of subscribing institutions was 40 in 1914, as compared with 30 in 1913, 29 in 1912, 25 in 1911, 24 in 1910, 20 in 1909, 18 in 1908, and 16 in 1907. The list is given on p. 356. Amherst College, Beloit College, Johns Hopkins University, Rutgers College, and the University of Wisconsin, are among the new subscribing institutions. The total membership of the Corporation is now 327 as against 303 in 1913. The receipts from subscribing institutions and students' fees were $7,300.00 as compared with $6,160.00 in 1913, $5,175.00 in 1912, $4,574.99 in 1911, $4,150.00 in 1910, and $3,700.35 in 1909. The receipts from the supply department were $14,003.35[1] in 1914, as compared with $14,554.90 in 1913, $13,966.35 in 1912, $10,303.61 in 1911, $9,300.58 in 1910 and $8,549.55 in 1909.

The main event of the year was the occupation and dedication of the new permanent laboratory building, the gift of the president of the board of trustees, Mr. Crane. It has satisfied the pressing need of space for investigation which has been felt so keenly in the past two or three years; it has also provided improved facilities so greatly needed for certain types of research; and the new library room contained in it has enabled us to

[1] The business transacted in 1914 was actually $1,100 more than in 1913, but collections were slow.

undertake a definite policy of library expansion. The building proved to be perfectly adapted for the purposes for which it was erected.

The exercises in dedication of the new building held on July 10 were attended by representatives of universities, members of the staff, the investigators and students working at the Laboratory, and by many friends. The buildings were open to inspection in the morning, and demonstrations of sea-animals and of research in progress were made, and the laboratory steamer made a collecting trip. Lunch was served to the invited guests. The formal exercises were held at 2 P.M., in a tent erected for the occasion, with an attendance of about 800. The addresses were interspersed with music by the Russian Balalaika Orchestra.

Mr. C. R. Crane, president of the Board of Trustees, and donor of the building presided. In opening the exercises, he said:

"I think we have come here particularly to celebrate the wonderful spirit that is back of the Woods Hole Biological Laboratory. It is very difficult to define that spirit, but I think we all know something of it and something is also known all through the scientific world. Without that spirit no amount of bricks and mortar and organization would be of any great service, but with that spirit the laboratory has been able to accomplish a very great deal with very simple means.

"For some time back it has seemed to be worth while to give this spirit a more substantial body. This spirit, as I see it, is very much like the spirit that President Wilson speaks so much of, the spirit of freedom and of coöperation, the fundamental spirit of democracy. In giving this spirit a more substantial body, we have been very fortunate in having with us Dr. Drew. I think we are all very happy at the wonderful result of his year's work. There is a rumor in circulation around here that Dr Drew is a zoölogist. I believe that rumor has spread into the outside world, but I am very certain that we must all feel, after looking over the new laboratory, that Dr. Drew would have made his reputation as an engineer if he had a chance."

Short speeches were made by the Director, by Professor Conklin, and by the head of the U. S. Bureau of Fisheries, the Hon. Hugh M. Smith. Dr. R. M. Woodward, the Director of

the Carnegie Institution of Washington, then delivered the main address on the Needs of Research. These addresses were published in full in *Science*.[1]

Extra provision was made this summer for the expected large attendance, by the lease of the Dexter House, which was run by the laboratory, and was made to pay expenses, including rent. With this increase the mess was able to accommodate the students and investigators comfortably. The Dexter House is in bad repair, and no lasting arrangement with the owner seems possible. We have therefore been casting about for other arrangements. The "Homestead" used for the help of the mess has long been overcrowded, and for some time we have regarded it as unsafe. Mr. Crane therefore offered to build on the homestead site a new dwelling house with accommodations for about forty people, which will be 10 to 12 in excess of the number employed in the Mess, and will therefore furnish some available space for women of the laboratory. This work is already nearly finished. Mr. Crane has also presented this autumn (1914) funds for improvement of the Cayadetta and for other purposes including the completion of the stone wall on the Yacht Club frontage and filling in behind it. This work is far advanced; when it is finished the building will be moved from in front of the new laboratory to the east end of the lot.

We shall thus begin the new year with most of our material needs satisfied to an extent that will probably be adequate for several years. The estimate of running expenses for 1915 shows a deficit of $20,000 above receipts, as in 1914. Mr. Crane has again most generously promised his support to this extent. This brings up again the need of an endowment, which I think we should keep constantly before us until attained. The flourishing condition of the Laboratory constitutes a strong argument for its endowment; the Laboratory represents no new experiment, but a demonstrated success, and the fulfilment of one of the greatest needs of American biology. The coöperation of forty American universities and the attendance of representatives of thirty-seven more proves that we are supplying a want that is felt by all the institutions of higher learning. The

[1] Vol. XL., No. 1024, pp. 217–232.

attendance of the largest body of scientific investigators ever gathered for work at one place and time also proves the magnitude of the want that we supply. Not only so but it demonstrates the great influence over the progress of research which the Marine Biological Laboratory exerts. We can feel justified in using all the means in our power to secure the funds that will place this great organization beyond the stress of ordinary vicissitudes.

Provision has been made for continuation of the students' courses as in 1914, with one exception. Dr. Drew has felt for some years that the burden of executive work during the summer is so great that it is undesirable for him longer to retain charge of the course in embryology. The directors have therefore requested Professor Wm. E. Kellicott, who has been associated with Dr. Drew in this course for several years, to assume charge of it. He has consented, and we may feel confident that he will maintain its best traditions. We must all feel nevertheless a sense of loss in the relinquishment by Dr. Drew of this important course. For many years in charge of the course in invertebrate zoölogy, and then of the course in embryology, Dr. Drew has impressed the lessons of our science on students to an extent which few teachers can equal. I am sure that all will join in congratulating Dr. Drew on his great success as a teacher, and in the hope that the future will yield him more leisure again to resume this cherished part of a scientist's work.

There are submitted as parts of this report lists of the staff of 1914, investigators and students in attendance, subscribing institutions, evening lectures and of members of the corporation.

1. THE STAFF

1914

FRANK R. LILLIE, DIRECTOR,

Professor of Embryology, and Chairman of the Department of Zoölogy, The University of Chicago.

GILMAN A. DREW, ASSISTANT DIRECTOR,

Marine Biological Laboratory.

ZOÖLOGY

I. INVESTIGATION

GARY N. CALKINS.....Professor of Protozoölogy, Columbia University.
E. G. CONKLIN.....Professor of Zoölogy, Princeton University.
GILMAN A. DREW.....Assistant Director, Marine Biological Laboratory.
GEORGE LEFEVRE.....Professor of Zoölogy, The University of Missouri.
FRANK R. LILLIE.....Professor of Embryology, The University of Chicago.
C. E. MCCLUNG.....Professor of Zoölogy, University of Pennsylvania.
T. H. MORGAN.....Professor of Experimental Zoölogy, Columbia University.
E. B. WILSON.....Professor of Zoölogy, Columbia University.

II. INSTRUCTION

CASWELL GRAVE.....Associate Professor of Zoölogy, Johns Hopkins University.
W. C. ALLEE.....Instructor in Biology, Williams College.
GEORGE A. BAITSELL.....Fellow in Zoölogy, Yale University.
RAYMOND BINFORD.....Professor of Biology, Earlham College.
E. J. LUND.....Bruce Fellow in Zoölogy, Johns Hopkins University.
T. S. PAINTER.....Graduate Student, University of Würzburg.

EMBRYOLOGY

I. INVESTIGATION (See Zoölogy)

II. INSTRUCTION

GILMAN A. DREW.....Assistant Director, Marine Biological Laboratory.
LORANDE L. WOODRUFF....Assistant Professor of Biology, Yale University.
WILLIAM E. KELLICOTT....Professor of Biology, Goucher College.
ROBERT A. BUDINGTON.....Professor of Zoölogy, Oberlin College.

PHYSIOLOGY

I. INVESTIGATION

ALBERT P. MATHEWS.....Professor of Physiological Chemistry, The University of Chicago.
R. S. LILLIE.....Professor of Biology, Clark University.
HAROLD C. BRADLEY.....Assistant Professor of Physiological Chemistry, University of Wisconsin.

II. INSTRUCTION

RALPH S. LILLIE.....Professor of Biology, Clark University.
WALTER E. GARREY.....Associate Professor of Physiology, Washington University Medical School.
FRANK P. KNOWLTON.....Professor of Physiology, Syracuse University.
EDWARD B. MEIGS.....Associate in Physiology, Wistar Institute of Anatomy and Biology.

PHILOSOPHICAL ASPECTS OF BIOLOGY AND ALLIED SCIENCES

LECTURES

EDWARD G. SPAULDING.....Assistant Professor of Philosophy, Princeton University.

BOTANY

GEORGE T. MOORE.....Director, Missouri Botanical Garden and Professor of Botany, Washington University.
GEORGE R. LYMAN.....Assistant Professor of Botany, Dartmouth College.
B. M. DUGGAR.....Physiologist, Missouri Botanical Garden and Professor of Plant Physiology, Washington University.
IVEY F. LEWIS.....Assistant Professor of Botany, University of Wisconsin.
W. J. ROBBINS.....Instructor in Plant Physiology, Cornell University.
A. R. DAVIS.....Lackland Research Fellow, Shaw School of Botany.

LIBRARY

H. MCE. KNOWER.....Professor of Anatomy, University of Cincinnati, Librarian.
MARY E. SCOTT.....Assistant Librarian.

CHEMICAL SUPPLIES

OLIVER S. STRONG.....Instructor in Anatomy, College of Physicians and Surgeons, New York City, Chemist.

SUPPLY DEPARTMENT

G. M. GRAY.....Curator.
JOHN J. VEEDER.....Captain.
E. M. LEWIS.....Engineer.
O. F. CURTIS.....Collector and Assistant Curator of Botanical Supplies.
A. W. LEATHERS.....Collector.
A. M. HILTON.....Collector.
EDNA E. WELLS.....Clerk

F. M. MACNAUGHT.....Business Assistant.

2. INVESTIGATORS AND STUDENTS

INVESTIGATORS

1914

ZOOLOGY

Independent Investigators

ALLEE, W. C., Assistant Professor in Zoölogy, University of Oklahoma.
BAITSELL, GEORGE A., Instructor in Biology, Yale University.
BEGG, A. S., Instructor in Comparative Anatomy, Harvard Medical School.
BINFORD, RAYMOND, Professor of Zoölogy, Earlham College, Richmond, Ind.
BROWNE, ETHEL N., 510 Park Ave., Baltimore, Md.
BUDINGTON, ROBERT A., Professor of Zoölogy, Oberlin College.
CALKINS, GARY N., Professor of Protozoölogy, Columbia University.
CHIDESTER, FLOYD E., Assistant Professor of Zoölogy, Rutgers College.
CHILD, C. M., Associate Professor of Zoölogy, University of Chicago.
CLAPP, CORNELIA M., Professor of Zoölogy, Mount Holyoke College.
CLARK, ELIOT R., Professor of Anatomy, University of Missouri.
CLARK, ELEANOR L., Johns Hopkins Medical School.
COE, WESLEY R., Professor of Biology, Yale University.
CONKLIN, E. G., Professor of Biology, Princeton University.
COWDRY, EDMUND V., Associate in Anatomy, Johns Hopkins Medical School.
COWDRY, N. H., Johns Hopkins Medical School.
CRAMPTON, H. E., Professor of Zoölogy, Barnard College, Columbia Univ.

DANCHAKOFF, WERA, Director of laboratory for medical research, Moscow, Russia.
DOLLEY, DAVID H., Professor of Pathology, University of Missouri.
DONALDSON, H. H., Wistar Institute of Anatomy and Biology.
DREW, GILMAN A., Assistant Director, Marine Biological Laboratory.
DUNN, ELIZABETH H., Woods Hole, Mass.
ERDMANN, RHODA, Theresa Leesell Research Fellow, Yale University.
GOLDFARB, A. J., Professor of Biology, College of the City of New York.
GRAVE, CASWELL, Associate Professor of Zoölogy, Johns Hopkins University.
HEGNER, ROBERT W., Assistant Professor of Zoölogy, University of Michigan.
HOGUE, MARY J., Instructor in Zoölogy, Wellesley College.
JACKSON, FREDERIC S., Lecturer in Histology and Embryology, McGill Univ.
JORDAN, HARVEY E., Professor of Histology and Embryology, University of Virginia.
KELLICOTT, W. E., Professor of Biology, Goucher College.
KINGSBURY, FRANCIS B., Instructor, University of Minnesota.
KNOWER, HENRY MCE., Professor of Anatomy, University of Cincinnati.
KUNKEL, BEVERLY W., Professor of Zoölogy, Beloit College.
LEFEVRE, GEORGE, Professor of Zoölogy, University of Missouri.
LEWIS, MARGARET R., Johns Hopkins Medical School.
LEWIS, WARREN H., Professor of Physiological Anatomy, Johns Hopkins Medical School.
LILLIE, FRANK R., Professor of Embryology, University of Chicago.
LOOMIS, FREDERIC B., Professor of Comparative Anatomy, Amherst College.
LUND, E. J., Instructor in Protozoölogy, University of Pennsylvania.
MACDOWELL, E. C., Instructor, Yale University.
MALONE, E. F., Assistant Professor of Anatomy, University of Cincinnati.
MCCLUNG, C. E., Director of the Zoölogical Laboratory, University of Pennsylvania.
MORGAN, T. H., Professor of Experimental Zoölogy, Columbia University.
NEWMAN, H. H., Associate Professor of Zoölogy, University of Chicago.
NOWLIN, NADINE, Assistant Professor of Zoölogy, University of Kansas.
PAINTER, T. S., Instructor, Yale University.
PATTERSON, J. T., Professor of Zoölogy, University of Texas.
PEARL, RAYMOND, Biologist of Maine Agricultural Experiment Station.
PEEBLES, FLORENCE, Lecturer, Bryn Mawr College.
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SHOREY, MARIAN L., Professor of Biology, Milwaukee-Downer College.
SPAULDING, E. G., Professor of Philosophy, Princeton University.
STOCKARD, CHARLES R., Professor of Anatomy, Cornell Medical College.
STRONG, O. S., Instructor in Anatomy, Columbia University.
STRONG, R. M., Instructor in Zoölogy, University of Chicago.
TENNENT, DAVID H., Professor of Biology, Bryn Mawr College.
WIEMAN, H. L., Assistant Professor of Zoölogy, University of Cincinnati.
WILDMAN, E. E., University of Pennsylvania.
WILSON, E. B., Professor of Zoölogy, Columbia University.
WOODRUFF, L. L. Assistant Professor of Biology, Yale University.
ZELENY, CHARLES, Associate Professor of Zoölogy, University of Illinois

Beginning Investigators

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ALLEN, EZRA, Professor of Biology at Philadelphia School of Pedagogy, University of Pennsylvania.
BRIDGES, CALVIN B., Fellow in Zoölogy, Columbia University.
BULLOCK, FREDERICK D., Instructor in Cancer Research, Columbia University.
CAROTHERS, E. ELEANOR, University of Pennsylvania.
COBB, MARGARET V., University of Illinois.
DEXTER, JOHN S., Graduate Student, Columbia University.
FIELD, HAZEL E., Graduate Student, University of Chicago.
GOODRICH, H. B., Fellow in Zoölogy, Princeton University.
GOULD, HARLEY N., Fellow in Biology, Princeton University.
GUNTHER, MAUDE C., Instructor in Biology, Eastern High School, Washington, D. C.
HEILBRUNN, L. V., Assistant in Zoölogy, University of Chicago.
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HOY, WILLIAM E., JR., Fellow in Biology, Princeton University.
KEY, J. A., Student, Johns Hopkins Medical School.
LARRABEE, AUSTIN P., Professor of Biology, Fairmount College.
LEATHERS, A. L., Instructor in Zoölogy, Northwestern University.
MEDES, GRACE, Graduate Student, Bryn Mawr College.
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MOORE, CARL R., Fellow in Zoölogy, University of Chicago.
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OKKELBERG, PETER, Instructor in Zoölogy, University of Michigan.
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SHUMWAY, WALDO, Assistant in Zoölogy, Columbia University.
STARK, MARY B., Graduate Student, Columbia University.
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WOODWARD, ALVALYN E., Fellow in Zoölogy, University of Michigan.
YOCUM, H. B., Instructor in Zoölogy, Kansas State Agricultural College.
YOUNG, DONNELL B., Laboratory Assistant, Columbia University.

PHYSIOLOGY

Independent Investigators

BRADLEY, H. C., Associate Professor of Physiological Chemistry, University of Wisconsin.
CHAMBERS, ROBERT, JR., Assistant Professor of Histology and Comparative Anatomy, University of Cincinnati.
GARREY, W. E., Associate Professor of Physiology, Washington University Medical School.
HARVEY, E. N., Instructor in Physiology, Princeton University.
HYDE, IDA H., Professor of Physiology, University of Kansas.
JUST, E. E., Professor of Physiology, Howard University.
KANDA, SAKYO, Research Assistant, University of Minnesota.
KITE, GEORGE D., Assistant in Physiology, Henry Phipps Institute.
KNOWLTON, FRANK P., Professor of Physiology, Syracuse University, College of Medicine.

LILLIE, R. S., Professor of Biology, Clark University.
LOEB, JACQUES, Head of Department of Experimental Biology, Rockefeller Institute for Medical Research.
MATHEWS, A. P., Professor of Physiological Chemistry, University of Chicago.
MATTILL, HENRY A., Professor of Physiological Chemistry, University of Utah.
MEIGS, EDWARD B., Fellow in Physiology, Wistar Institute of Anatomy and Biology.
MOORE, A. R., Associate Professor of Physiology, Bryn Mawr College.
OLIVER, WADE W., Student, Ohio-Miami Medical College.
TASHIRO, SHIRO, Instructor in Physiological Chemistry, University of Chicago.
UHLENHUTH, EDUARD, Rockefeller Institute for Medical Research.
WARREN, HOWARD C., Stuart Professor of Psychology, Princeton University.
WASTENEYS, HARDOLPH, Associate in Experimental Biology, Rockefeller Institute for Medical Research.
WERBER, ERNEST I., Instructor in Anatomy, Northwestern University.
WHERRY, W. B., Associate Professor of Bacteriology, University of Cincinnati.

Beginning Investigators

HYMAN, LIBBIE H., Laboratory Assistant in Zoölogy, University of Chicago.

BOTANY

Independent Investigators

BLAKESLEE, A. F., Professor of Botany and Genetics, Connecticut Agricultural College.
COLLEY, R. H., Instructor in Botany, Dartmouth College.
DAVIS, A. R., Lackland Research Fellow, Shaw School of Botany, Washington University.
DUGGAR, B. M., Physiologist in charge of Graduate Laboratory and Professor of Plant Physiology, Missouri Botanical Garden.
GATES, R. R., University of London, England.
LEWIS, I. F., Professor of Botany, University of Missouri.
LYMAN, GEORGE R., Assistant Professor of Botany, Dartmouth College.
MOORE, GEO. T., Director, Missouri Botanical Garden.
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Beginning Investigators

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WUIST, ELIZABETH D., Instructor in Botany, Milwaukee State Normal School.

STUDENTS

1914

ZOOLOGY

ALLEN, WILLIAM RAY, Assistant in Kansas State Agricultural College, Manhattan, Kans.
APPLEGATE, ANNE G., Student, Western College, Oxford, Ohio.

APPLEGATE, ELEANOR, Western College, Oxford, Ohio.
ASHMAN, RICHARD, Student, Rutgers College, New Brunswick, N. J.
BALLOU, MARION M., Mount Holyoke College.
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GREENE, WALTER F., Amherst College.
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HAMILTON, FLORENCE N., Student, Vassar College.
JACKSON, FRANCES E., Student, Mt. Holyoke College.
JANNEY, MARION, Goucher College.
KELLOGG, EMILIE, Student, Mt. Holyoke College.
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RUSH, J. E., Assistant Professor, Wisconsin.
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STRONGMAN, BESSIE T., Student, University of Colorado.
THOMAS, ANNA M., Student, Carnegie Institute of Technology.
TIESING, PAUL E., Student, Yale University.
WARREN, CATHERINE C., Princeton, N. J.
WAYMAN, MARGUERITE, Student, Hunter College, New York City.

EMBRYOLOGY

ALLEN, CHARLES E., Assistant Instructor, Wabash College.
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BINKLEY, LELIA T., University of Texas.
CHAMBERLAIN, MARY M., Student, Bryn Mawr College.
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DIEHL, JANE K., Student, Wellesley College.

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HUNTER, OSCAR B., Professor of Histology and Embryology, Associate in Anatomy, George Washington Univers ty, Washington, D. C.
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MCFARLAND, HELEN J., Student, Bryn Mawr College.
MILLS, FRANCES A., Barnard College.
NELSON, THURLOW C., Assistant in Zoölogy, University of Wisconsin.
OGDEN, WARNER, Student, Carleton College, Northfield, Minn.
PLOUGH, HAROLD H., Assistant in Zoölogy, Amherst College.
POTTER, BESS, Student, Doane College, Crete, Neb.
PREBLE, JESSIE L., Student, Bryn Mawr College.
TAFT, ANNIE E., Curator Dept. of Neuropathology, Harvard Medical School.
WALTON, ARTHUR C., Student Assistant, Northwestern University.
WARE, CLARA C., Graduate Student, Columbia University.
YOUNG, THOMAS O., Student, Carleton College, Northfield, Minn.

PHYSIOLOGY

ATWOOD, WARREN G., Student, Dartmouth College.
BENJAMIN, BLANCHE M., Student, Radcliffe College.
FREDERICK, NORA, Teacher in Biology, Lewis Institute, Chicago.
HYMAN, LIBBIE H., Laboratory Assistant in Zoölogy, University of Chicago.
LINTON, EDWIN S., Graduate Student, Washington and Jefferson College.
LYNCH, VERNON, Graduate Student, Johns Hopkins University.
OLIVER, SYMMES F., University of Michigan, Ann Arbor.
REDFIELD, ALFRED C., Assistant in Zoölogy, Harvard University.
VANNEMAN, AIMÉE S., Vassar College.
WALLING, LALIA V., Instructor in Physiology, University of Kansas.

BOTANY

ALLARD, ANNE D., Teacher in Boston Normal School, Boston.
BURKS, GEORGE PAUL, Student, Wabash College.
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FINE, SOLOMON, Student, Rhode Island State College, Kingston, R. I.
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GREEN, NEWTON B., Student, Oberlin College.
HERRICK, JOSEPH C., Professor of Biology, St. Joseph's Seminary, Yonkers, N. Y.
MALLARD, AGNES K., Teacher, Boston Normal School, Boston.
MCLAUGHLIN, FREDERICK A., Instructor in Botany, Mass. Agricultural College.
SEVERY, J. WARREN, Student, Oberlin College.
STEARNS, FRANCES L., Teacher, Central High School, Grand Rapids, Mich.
WANN, F. B., Missouri Botanical Garden, St. Louis, Mo.
WILLEY, ALBERT G., Student, Dartmouth College.

3. TABULAR VIEW OF ATTENDANCE

	1911	1912	1913	1914
INVESTIGATORS—Total	82	93	122	128
Independent:				
Zoölogy	42	44	58	62
Physiology	18	14	17	22
Botany	8	10	11	10
Under Instruction:				
Zoölogy	12	21	21	31
Physiology	2	2	7	1
Botany		2	7	3
STUDENTS—Total	65	67	69	89
Zoölogy	26	24	33	43
Embryology	20	15	22	21
Physiology	6	11	8	10
Botany	13	17	7	15
TOTAL ATTENDANCE	147	160	191	217
INSTITUTIONS REPRESENTED—Total		57	80	77
By investigators	37	43	50	51
By students	31	36	41	47
SCHOOLS AND ACADEMIES REPRESENTED				
By investigators	3	2	3	1
By students	9	1	6	5

4. SUBSCRIBING INSTITUTIONS

AMHERST COLLEGE.
BARNARD COLLEGE.
BELOIT COLLEGE.
BRYN MAWR COLLEGE.
CARLTON COLLEGE.
COLUMBIA UNIVERSITY.
CARNEGIE INSTITUTE OF TECHNOLOGY.
DARTMOUTH COLLEGE.
DOANE COLLEGE.
GOUCHER COLLEGE.
HARVARD UNIVERSITY.
HARVARD MEDICAL SCHOOL.
HUNTER COLLEGE, N. Y. C.

KANSAS STATE AGRICULTURAL COLLEGE.
JOHNS HOPKINS UNIVERSITY.
LUCRETIA CROCKER SCHOLARSHIPS.
MCGILL UNIVERSITY.
MOUNT HOLYOKE COLLEGE.
NORTHWESTERN UNIVERSITY.
OBERLIN COLLEGE.
PRINCETON UNIVERSITY.
RADCLIFFE COLLEGE.
RHODE ISLAND STATE COLLEGE.
ROCHESTER UNIVERSITY.
ROCKEFELLER INST. FOR MED. RESEARCH.
SMITH COLLEGE.
RUTGERS COLLEGE.
UNIVERSITY OF CHICAGO.
UNIVERSITY OF CINCINNATI.
UNIVERSITY OF ILLINOIS.
UNIVERSITY OF KANSAS.
UNIVERSITY OF MICHIGAN.
UNIVERSITY OF PENNSYLVANIA.
UNIVERSITY OF WISCONSIN.
VASSAR COLLEGE.
WELLESLEY COLLEGE.
WESTERN COLLEGE.
WISTAR INSTITUTE.
WABASH COLLEGE.
YALE UNIVERSITY.

5. EVENING LECTURES, 1914

Friday, July 3,
PROF. T. H. MORGAN....."Chromosomes and Mendelian Heredity."

Tuesday, July 7,
DR. ERWIN F. SMITH....."Crown Gall in Plants, with Reference to the Nature and Origin of Cancer."

Tuesday, July 14,
DR. G. L. KITE....."The Colloidal Structure of Living Matter as Determined by Micro-dissection."

Friday, July 17,
DR. LAWRENCE J. HENDERSON.. "The Functions of the Environment."
Tuesday, July 21,
PROF. D. H. TENNENT..... "Hybridization in Sea-urchins."

Friday, July 24,
PROF. H. C. WARREN..... "Freedom of Teaching in American Colleges."
Tuesday, July 28,
DR. FRANK M. CHAPMAN..... "An Ornithological Expedition to Colombia."
Friday, July 31,
DR. RAYMOND PEARL..... "The Physiology of Reproduction in the Domestic Fowl."
Tuesday, Aug. 4,
DR. ALFRED G. MAYER..... "The Coral Reefs of Torres Straits."
Friday, Aug. 7,
DR. EDWIN LINTON..... "Reminiscences of the Woods Hole Laboratory of the U. S. Fish Commission, 1882–1889."
Friday, Aug. 14,
DR. R. R. GATES..... "Recent Aspects of Mutation."

6. MEMBERS OF THE CORPORATION.

1. LIFE MEMBERS

ALLIS, MR. E. P., JR., Palais Carnoles, Menton, France.
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CONKLIN, PROF. EDWIN G., Princeton University, Princeton, N. J.
CRANE, MR. C. R., Woods Hole, Mass.
DAVIS, MAJOR HENRY M., Syracuse, N. Y.
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KIDDER, MR. NATHANIEL T., Milton, Mass.
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MASON, MISS IDA M., 1 Walnut St., Boston, Mass.
MEANS, MR. JAMES HOWARD, 196 Beacon St., Boston, Mass.
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NUNN, MR. LUCIAN L., Telluride, Colo.
OSBORN, PROF. HENRY F., American Museum of Natural History, New York.
PHILLIPS, DR. JOHN C., Windy Knob, Newham, Mass.
PHILLIPS, MRS. JOHN C., Windy Knob, Newham, Mass.
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WILLCOX, MISS MARY A., Wellesley College, Wellesley, Mass.
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WILLIAMS, MRS. ANNA P., 505 Beacon St., Boston, Mass.
WILSON, DR. E. B., Columbia University, New York City, N. Y.
WILSON, PROF. W. P., Philadelphia Museum, Philadelphia, Pa.

2. MEMBERS, JANUARY, 1915

ABBOTT, PROF. J. F., Washington University, St. Louis, Mo.
ABBOTT, MISS MARGARET B., The Bennett School, Milbrook, N. Y.
ADDISON, DR. W. H. F., University of Pennsylvania, Medical School, Philadelphia, Pa.
ADKINS, MR. W. S., Texas Christian University, Fort Worth Texas.
ALLEE, DR. W. C., University of Oklahoma, Norman, Okla.
ALLEN, PROF. EZRA, 413 Lancaster St., Ardmore, Pa.
ALLYN, MISS HARRIET M., Hackett Medical College, Canton, China.
ALSBURG, DR. C. S., U. S. Dept. of Agriculture, Washington, D.C.
BAITSELL, DR. GEORGE A., Sheffield Scientific School, Yale University, New Haven, Conn.
BAKER, DR. E. H., 5436 University Ave., Chicago, Ill.
BANCROFT, PROF. F. W., Aloha Farm, Concord, California.
BARDEEN, PROF. C. R., University of Wisconsin, Madison, Wis.
BECKWITH, MISS CORA J., Vassar College, Poughkeepsie, N. Y.
BEHRE, MISS ELINOR H., Sophie Newcomb College, Tulane University, New Orleans, La.
BEYER, DR. H. G., Stoneleigh Court, Washington, D. C.
BIGELOW, PROF. M. A., Teachers College, Columbia University, New York City, N. Y.

BIGELOW, PROF. R. P., Mass. Institute of Technology, Boston, Mass.

BINFORD, DR. RAYMOND, Earlham College, Richmond, Ind.

BINKLEY, MISS LELIA T., University of Texas, Austin, Texas.

BLAKESLEE, PROF. A. F., Connecticut Agricultural College, Storrs, Conn.

BOX, MISS CORA MAY, University of Cincinnati, Cincinnati, Ohio.

BRADLEY, DR. HAROLD C., University of Wisconsin, Madison, Wis.

BROWNE, MISS ETHEL N., East Hall, Univ. of California, Berkeley, Cal.

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CHESTER, PROF. WEBSTER, Colby College, Waterville, Me.

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CHILD, PROF. C. M., University of Chicago, Chicago, Ill.

CLAPP, PROF. CORNELIA M., Mount Holyoke College, South Hadley, Mass.

CLARK, DR. E. R., University of Missouri, Columbia, Mo.

COE, PROF. W. R., Yale University, New Haven, Conn.

COLLEY, DR. R. H., Dartmouth College, Hanover, N. H.

COLTON, PROF. H. S., Ardmore, Pa.

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COPELAND, DR. MANTON, Bowdoin College, Brunswick, Maine.

COWDRY, DR. E. V., Johns Hopkins Medical School, Baltimore, Md.

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CRANE, MRS. C. R., Woods Hole, Mass.

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DORRANCE, MISS FRANCES, Dorranceton, Pa.

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FIELD, PROF. IRVING, Auburn, Mass.

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HEATH, PROF. HAROLD, Stanford University, San Francisco, Cal.
HEGNER, PROF. R. W., University of Michigan, Ann Arbor, Mich.
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REGENERATIVE POTENCIES OF DISSOCIATED CELLS OF HYDROMEDUSÆ.

CHAS. W. HARGITT.

INTRODUCTORY.

At various times during my earlier work on the development and regeneration of hydromedusæ, especially that dealing with the developmental capacity of egg fragments, there had grown the conviction of the remarkable potencies of the various tissue elements of these organisms. So strong had this impression become that the desirability of repeating Trembly's picturesque experiment of turning *Hydras* inside out and testing again the possibilities that ectoderm and entoderm might really exchange functions under the new conditions involved was entertained. While admitting the rather convincing results of the experiments of Ischikawa ('90), touching this feature it still seemed that there might be some warrant that under certain conditions Trembly's conclusions might find confirmation. However, the writer never found the convenient season for trying out the experiment, though other correlated features were observed at various times in connection with the work above cited, especially in the summer of 1908 while working on the development of *Clava* and *Hydractinia*. This was further incited by the work of H. V. Wilson, "On Some Phenomena of Regeneration in Sponges," '07. Therefore with the opportunity for investigation at the Naples Laboratory I set about a series of experiments with a view to settle some of the problems concerned. My work at Naples began in December, 1910, and continued till mid-April following, and during this period systematic experiments were made upon the regenerative potencies of somatic cells of about a dozen different species, among which the following may be named: two species of *Eudendrium*, two species of *Tubularia*, one each of *Hydractinia*, *Podocoryne*, *Campanularia*, *Obelia*, *Halecium*, *Sertularia*, and a medusa, *Liriope exigua*.

In *Science* of March 10, 1911, appeared a preliminary report

of similar experiments by Dr. Wilson, which reached Naples about April 1, just when my own experiments were being concluded, results of which had already been written and some of which will appear in the following sections just as originally prepared. I immediately wrote Professor Wilson, giving a brief account of my work and stating that my results would be held pending the appearance of his completed report. This appeared in due course ('11). While dealing in the main with different material yet he had employed essentially the same methods which I had followed and they seemed so conclusive that I had laid my own paper aside, deeming it unnecessary. However, the report in the *Journal* of the Marine Biological Association of similar experiments by DeMorgan and Drew (Oct., '14) which seemed to express some doubt as to the conclusiveness of Wilson's results, prompts me to submit even at this late date my own results, though in somewhat abbreviated form.

Material and Methods.

Concerning material employed in the experiments mention has been made in the previous section as to the several species used, though two species of medusa instead of one were experimented upon. For the most part particular care was taken to have perfectly fresh and vigorous specimens, but it was later found that this precaution was not absolutely essential in all cases, some of my best hydranths having been reared from material which had been several days in the laboratory before being used. Later mention will also be made of a probable reason why this may happen. One point however calls for special emphasis, namely, that of the freshness and purity of the water used in the experiments. In my work several expedients were employed to guard against the presence of parasitic organisms, especially predatory protozoa. Of most efficiency was that of having water fresh from the open sea. Another expedient was that of sterilizing water of the aquaria, and still a third was that of using synthetic, that is, artificial sea-water. But of all these the first was found to be most satisfactory.

Concerning methods much might be said, though only the briefest reference will be made of those employed by me. Among

the several modes of isolating the tissue cells the following were employed: With hydroids the cœnosarc may be forced out of the perisarc by clipping off the hydranths and then stripping the stems through clean fingers, or the ends of smooth forceps, or similar device. Again, one may finely clip up the stems with scissors and then still further reduce the cells by continuing the operation in deep watch glasses or small beakers. A still further mode was used, that of clipping up the stems with scissors and later grinding the tissues under a smooth glass rod whose end had been rounded in the flame, employing it as a pestle and a watch glass as a mortar. One objection to this was the crushing of the individual cells in many instances, and otherwise injuring them. On the other hand there may be reason to believe that such treatment is not really so serious an injury as might at first sight appear, for as will be seen in some later discussion, the shock may actually serve as a stimulus to hasten cellular de-specialization and hence initiate regenerative processes.

Cell dissociation having been effected the next method is to arrange them in dishes where aggregation may take place. First in this operation is the straining or filtering out of debris and such fragments as are undesirable. This was done chiefly by pressing the cells through bolting cloth. In my experiments it was found that a better medium than silk bolting cloth was a fine meshed cheese cloth, or a coarse meshed linen or cotton fabric, which was softer and apparently more efficient. In a few cases in the earlier experiments I merely placed the entire mass in watch glasses and with a pipette carefully drew off most of the coarser stuff and left the cells as free as such a process might leave them. On the whole, the pressing process worked better and was more expeditious. Following this operation the cells were left for a time to settle and then the milky sediment was carefully drawn off, when fresh water was added and the dishes set aside in bowls surrounded by running water to ensure as constant a temperature as could be had.

The Experiments.

Podocoryne carnea.—This was the first species which came to hand and proved one of the most responsive and convincing

of the entire series. Several colonies were brought in, all occupying shells inhabited by hermit crabs, the usual habitat of this species. In one respect the species is rather difficult to operate on owing to the spinous condition of the basal cœnosarc, which made it hard to obtain enough of the polyps to make the desired culture. By allowing the colony to expand fully in shallow dishes it was possible by a dextrous sweep of the scissors to cut off quite a bunch at a single time, and by allowing others to expand in the same way and repeat the operation it was found possible to secure sufficient material for the culture. The specimens were finely cut or ground into a pulpy mass, filtered through the sterilized cloth, and thus fitted to undergo later changes. It may as well be stated here that among hundreds of preparations relatively few gave completely successful results in the regeneration of new polyps. My first surprise was not that many of the preparations "went bad," but that any survived the operation and went forward in regeneration. Here as in most experiments on regeneration a large mortality occurs in the preparations.

Character of the Dissociated Cells.—If examined soon after their dissociation one may easily distinguish the several sorts of cells even under a magnification of three hundred diameters, that is, ectoderm, entoderm, nematocyst, interstitial, etc. The very minute ectoderm cells are in striking contrast with the large flagellated cells of the entoderm. In the course of an hour, sometimes less, these differences become less marked, and ultimately almost disappear. They have become despecialized into potentially embryonic cells, and probably from this change have acquired their regenerative capacities. A careful study of such dissociated cells from various species has strongly suggested the probability that some such cytomorphic process is involved in most regenerative phenomena, and leaves little doubt that the features under consideration here are positively brought about through such a process.

Cell Aggregation.—Examination of a culture within a few hours, three to five, will show that a remarkable change has taken place among the cells in their relations to each other. They will be found to have formed numerous small nodular groups

having the appearance in many cases of embryonic morulæ, or blastulæ. It was this phenomenon among others already mentioned which first raised the question in my mind as to their regenerative possibilities many years ago. Concerning the mode by which this process of aggregation is brought about there is some doubt. The attempt was made to actually observe it by carefully keeping a fresh culture under direct observation with the microscope. It was thought that the action of the flagellated cells of the entoderm might act as a means by causing vortices in the water, but careful study failed to show that this was a factor of any direct value. Such action of these cells may be easily seen but its effects are as often repellent as attractive. The fact that certain of the cells show amœba-like aspects suggested a possible amœboid action in the process. But here again no evidence whatever was found to prove the suggestion. One might imagine some chemotropic influence, but no evidence was found that such was the case. I am inclined to the view that chance contact is perhaps the chief factor in the process. This is made probable by the fact that such aggregation may be greatly facilitated by mechanical agitation of the cells, and by a gentle rotary motion of the dishes. In the earlier experiments considerable care was taken to handle the dishes as little as possible during the early stages of an experiment, thinking such might be undesirable, but later the opposite view was taken, and the dishes often rotated to hasten the process. It must be admitted, however, that there seemed to be other factors involved, for even when a considerable mass of cells had been brought together by this means there was later found to have been a sort of segregative process at work, for the mass had been more or less broken up into sections or lobes which later behaved as entirely independent bodies.

The cell aggregates, while rather predominantly sub-spherical in shape, showed considerable variation. Some were flattish, or disk-like, and some were somewhat lobulated and irregular in shape. But throughout a series of such aggregates one of the most conspicuous features was that already referred to above, namely, the resemblance to an embryonic blastula or morula, especially a hydroid morula; and if one were to take account of

such morulæ as those of *Pennaria* or *Turritopsis* or *Hydractinia* it would include practically the entire range of shape exhibited by these regeneration aggregates, and one might designate them as regeneration morulæ, for such they really seem to be.

Encystment.—Following the process of aggregation there occurred in those vitally active a process of encystment, that is, the secretion of a definite perisarc about the entire mass, and its adhesion to the bottom of the glasses. Lest this feature be regarded as peculiar to these particular cases it should be pointed out that the phenomenon is often shown at a certain stage in the normal development of the hydromedusæ, and indeed in some scyphomedusæ as well. The writer has directed attention to this in the case of *Cyanea* ('02, '10) and it is doubtless shared by many others. Its function is doubtless protective, just as is that of the perisarc in the adult hydroid. Encystment usually occurs shortly following the completion of the phase of aggregation just described. This encysted stage may continue for an indefinite time, or it may be of short duration. The latter was more frequently the case with *Podocoryne* than with some others. In the present case the cyst was frequently ruptured for the upgrowth of the hydranth within a comparatively short time, say two days; but in many cases this stage persisted for a week or even more, and indeed in certain cases the cyst became a prison, being so dense as to become impenetrable from within as well as without. This again is comparable with what may happen in such stages in normal development (vide supra). This process of perisarc formation often takes various forms, following the phases of growth. In *Podocoryne* there was frequently the development of a reticulated hydrorhiza before the appearance of a hydranth, and later there appeared nodular enlargements of these stolon-like tubes and from these points would occur the upgrowth of a series of polyps. In one such preparation I obtained three vigorous young hydranths.

What has been stated in this connection as to *Podocoryne* is likewise true of other species experimented with. The behavior of the encysted aggregation morulæ is quite like that of the growing stolons of the hydrorhiza. Both may live for weeks under these conditions without any signs of further development.

Again, after such prolonged periods there may come about another direction of regenerative activity and a hydranth may arise. Aside from the evidence of life observed in the active circulation within the cœnosarc of stolons it becomes easy for one to recognize in the character of the cells of the various structures the evidences of life or death, and furthermore death of any portion is rapidly followed by disintegration brought about by microörganisms. It should have been stated in the earlier part of this section that the process of encystment usually begins soon after the aggregation phase is complete, which may be within twelve to twenty hours, though it may not become evident until much later, thirty to forty hours. The first evidence of its formation is the adhesion of the mass to the bottom of the glass, and somewhat later may be distinguished as a very delicate transparent film covering the entire mass. Its later extension may be easily followed as the growth of stolons takes place, which may be quite rapid in some cases, or in others very slow. Here again as was pointed out in an earlier connection, there is a marked similarity in the aspects of regenerative growth and those of embryonic development which further emphasizes the probability that they are fundamentally identical, having their initiative in potentially embryonic cells.

Polyp Formation.—In *Podocoryne* the first evidence of definitive hydranth organization was found during the second day following the experiment. This consisted in the dissolution of the cyst at its upper surface and the protrusion of a bud-like upgrowth. At first these were barely distinguishable, but during the third day they had become large enough to be seen with the unaided eye. The first fully formed hydranth appeared early on the fifth day, when a polyp having the distinctive form of hypostome and three tentacles was noted. This was followed by further growth of the young specimen in all its parts. The movement of the tentacles and their growth in length was interesting and striking, leaving not the least doubt as to the genuineness of the regenerative process. Usually the first three tentacles appeared at about the same time, but in a few cases it was noted that when first observed there were but two, though a third appeared rather soon after. The full six tentacles of the new polyp were

developed within the next two or three days, and conformed exactly to the phases of the growth of an embryonic specimen. It should be emphasized in this connection that in the rate of growth in these specimens, as in the entire regenerative process, there was great individual difference. Apparently this was dependent upon the state of vitality of the underlying organization. For example, it was found that development was slower, and the resulting polyps smaller when arising from small cell aggregates, and in cases where there had been an excessive stolonization prior to polyp formation. In the one case it would seem as if the store of energy was small to begin with, and in the second that it had been depleted by excessive stolon formation.

The young polyps continued to live for several weeks, much longer than would have seemed probable when the highly artificial conditions, and the very limited food supply are taken into consideration. During the course of the experiments more than a dozen of these polyps of *Podocoryne* were reared to functional maturity and many others to such stage as to leave no trace of doubt as to the validity of the results.

Let it be remarked here that in this species all the material was in the asexual condition, that is, there were polyps only, no signs whatever of medusæ, which are the sexual stage in the life cycle of *Podocoryne*. Other experiments go to show that so far as use of material of asexual or mixed condition no difference as to regenerative potency could be distinguished. In *Eudendrium* where the medusa stage is absent, and where one finds sex cells in various stages of growth, the experiments were apparently not thereby influenced at all. Indeed, in those cases in which egg cells were present they took no part whatever in later regenerative activity, either degenerating or being absorbed as yolk material.

Eudendrium.—In experiments upon *Eudendrium* two species were used, *E. rameum*, and *E. racemosum*, both very common at Naples. Methods of treatment were the same as in the case already described. The promptness with which these hydroids had responded in the numerous previous experiments by the writer[1] and others in regeneration and regulation led me to

[1] Biol. Bull., Vol. I., p. 35.

anticipate that a similar type of reaction might be anticipated in this connection, but as will be seen this expectation was not realized fully. The early reactions in aggregation, encystment, etc., were quite as prompt and promising as in *Podocoryne*. And in these features the species showed nothing peculiar. But beyond the initial stages the results were disappointing. The mortality was much greater and the growth reactions much less energetic. Experiments were varied in every way practicable, hydranths alone being used for obtaining disorganized cells, cœnosarc alone, male colonies alone and female colonies alone. There seemed to be no very marked differences in results, though the cells obtained from crushing hydranths gave the least satisfactory results. As already stated the early stages followed quite as in *Pocodoryne*, encystment, and stolonization, but beyond these my experiments were far less satisfactory than in the former. In only a few cases was I able to obtain polyps, and these were small and very weak. A few developed tentacles, but never the usual number, nor were they more than buds on the base of the hydranth. The few polyps which developed secreted the usual perisarc, which was indistinguishable from that of an embryonic *Eudendrium*.

Tubularia.—As in the former I employed two species, *T. mesembryanthemum*, and *T. larynx*. As in the former the early reactions were prompt and quite like the others. But unlike the others my experiments never afforded a single polyp. The massing of dissociated cells was quite as prompt and the resulting morula-like embryo as promising as in either of the others. The encystment of perisarc followed in due order, and these lived for many days, but they never showed further signs of development. Perhaps no hydroid genus has had so large a place in experimental work as has *Tubularia*. If therefore my anticipations as to the behavior in cellular regeneration of *Eudendrium* were disappointing, those concerning *Tubularia* were really perplexing, at least for the time being. I think an explanation may be ventured which, though not absolutely convincing, may relieve a measure of the perplexity. It is to the effect that regenerative potency in an organism is more or less conditioned by its state of vitality, or in still more suggestive phrase, its *physiological*

state, at the time it is subjected to the test. This has been recognized in principle in experiments on ordinary regenerative processes, in that only especially vigorous specimens are used. Further discussion of this point will be deferred to another section of the paper.

Other Species.—As indicated in the outstart, about a dozen different species were tried in the course of the investigation. Among these were several campanularian hydroids and two species of medusæ. The hydroids tested gave the same initial responses as those just described for *Tubularia*, but beyond that the results were likewise negative. In all cases the phase of cell-aggregation was essentially the same as in the former cases. The same was likewise true of the internal organization of the morula-like embryo, and in the perisarc formation, but beyond this there was no development.

Species of sertularian hydroids and also of *Halecium* were tested and gave exactly the same initial responses, including encystment of the embryonic mass which lived for a time but soon showed signs of disintegration and death. The reactions of the last species were the least satisfactory of any tested.

Medusæ.—Two species of medusæ were tried, though with hardly any hope of getting any regenerative responses. They were prepared just as had been the hydroids, strained through the bolting cloth and set aside after addition of fresh water. An examination of the dissociated cells showed about the same condition of the other preparations, and further inspection in about an hour showed a series of the most beautiful cell-aggregates found in any of the experiments. When it is recalled that Medusæ represent the most highly specialized group of Hydrozoa it will seem strange to find cells thus organized after having been dissociated in the manner indicated. In all my observations upon cœlenterate development I have seldom seen more typical blastula-like embryos than those under review. Unless one were actually aware of their source he could hardly have been convinced that they were not genuine embryos in process of development. However, so far as my experiments show the regenerative process does not go farther. Moreover, the organism thus formed is very short lived, and devoid of further significance so far as our problem is concerned.

Addenda and Discussion.

As mentioned in the introduction the immeditae occasion leading to the publication of this paper at this time after having been laid aside for four years was the appearance in the *Journal* of the Marine Biological Association, October, 1914, of a paper by DeMorgan and Drew, setting forth the results of similar experiments, all of which had given generally negative results. Moreover, certain of their conclusions seemed to leave a measure of doubt concerning the conclusiveness of certain of Wilson's experiments, and phases of their discussion involved assumptions which are at variance with those which my own work had rendered very convincing.

In the first place I desire to refer briefly to Wilson's methods and results with most of which my own are in accord. His experiments on *Eudendrium* seem to have been much more successful than my own, for which I am very glad, since it confirms with great certainty points which in my own experiments were incomplete, though sufficiently complete to warrant definite conclusions. In another point Wilson's work goes beyond my own, namely, in the admirable demonstration which his actual sections of various stages affords as to the precise features involved in the regenerative process at given times. Furthermore, the excellent series of drawings and photographs illustrating his results leave nothing to be desired in that respect, and I am purposely omitting any of my own, the only series of which not better covered are those relating to *Podocoryne*, and in these nothing essentially different occurs.

The work of DeMorgan and Drew covered experiments on two species of *Antennularia* and are restricted to these only. In order to consider certain of their views it may be well to first quote certain specific statements in their own words. "Our results largely bear out his (Wilson) contentions, though we were not successful in carrying the regenerative process as far as the production of new hydranths, and the histological structure of the restitution masses we obtained differed in many ways from that described in Wilson's paper. These differences are probably due to the fact that we experimented with other species of hydroids to those used by Wilson. The especial interest of

our investigations lies in the rather anomalous fact that we have not been successful in obtaining regeneration of the complete organism from the dissociated cells. In our experiments the restitution masses, by some rearrangement or metaplastic process taking place among their conglomerated cells, formed tissue aggregates histologically reduplicating the structure of the parent organism, but in a *quite irregular and apparently meaningless manner*."

Two features in this quotation call for brief consideration, that included in the first sentence, and that which I have italicized in the last. It will have been noted in the accounts given in the earlier sections of this paper that I have given a number of cases comprising the exact equivalent of the failure they mention. This point will be further noted in a later paragraph. To the second feature it is only necessary to state that in normal hydroid development the *entire process is often "quite irregular and apparently meaningless,"* frequently more so than they found in the cases concerned. In a final paragraph the authors say: "Our experiments have resulted in the production of masses that are *certainly abnormal and pathological*, but nevertheless we would submit that the segregation and rearrangement of the cells after isolation, and the comparatively long duration of life of the tumor-like masses to which they give rise are facts of considerable theoretical interest."

In this quotation I have italicized the points to which it seems necessary to make some reference. It may be admitted that in some sense such restitution masses are *abnormal*, in that the very process by which their dissociation was brought about was presumably abnormal. But that the resulting restitution masses, involving as they have the regenerative potencies of the component cells, is abnormal I must seriously challenge. Again, the assumption that they are *pathological* I should emphatically doubt. The writer once submitted a series of preparations of embryological material to a well-known cytologist and received the (at that time) very disconcerting comment, "your preparations appear to have been made from pathological material." Yet from that very material I had been getting living embryos by the hundred! So in the present case to designate as pathological

cell aggregates which are producing right along perfectly normal and healthy polyps is to use a term whose significance implies the very opposite. It is admitted in the above citation that the tumor-like masses continued to live for a long time, as much as sixty days according to a preceding sentence, which shows a degree of vitality greater than that of colonies of the hydroid when placed in the aquarium. This fact of itself should prompt serious hesitation as to an assumption of a pathological condition.

It may throw some light upon the problem if attention is directed to conditions involved in the life history of many of these organisms. It is well known that many hydrozoa have alternating periods of activity and repose—growth, reproduction, etc., followed by corresponding periods of decline and more or less degeneration. In some these periodic alternations are correlated with seasonal changes in which temperature is an important factor. In others it is directly correlated with reproductive activities and has apparently little relation to season or temperature. What is of immediate importance in this connection is the fact of rather evident degenerative phases. For example, it is well known that in the spring, following the active reproductive period in several species of *Tubularia*, there is a marked degenerative phase, first evident in the casting off of the hydranths of almost the entire colony, then the gradual disintegration of the whole trophosome, till within a period of a few weeks it is difficult to find an entire and vigorous vegetative colony. An examination of the histological condition of the degenerative cœnosarc reveals the fact of positive decline marked by cytolytic conditions which might really be designated as pathologic for the time being. But even here a continued study would probably reveal the fact of its being associated with perfectly normal cyclic phases of life, being in fact phases of varying *physiological states* to which reference has already been made in an earlier connection. Similar facts of degeneration phases have also been described as associated with regenerative activity in hydroids. In experiments on *Tubularia* Stevens states in so many words: "The red granules seen in the circulation of regenerating pieces of *Tubularia* are derived from the disintegrating entodermal ridges, and are ejected by the young hydranth soon after

it emerges from the tube. They are waste material rather than formative substance" ('01, p. 414). The writer himself has made similar observations in several cases, and has demonstrated the regressive condition in the cœnosarc of hibernating specimens.

A most interesting study of a series of changes of apparenly-similar character has been made by Schultz, "Über Hungererscheinungen bei *Hydra fusca*" ('06), discussed under the larger topic of "Reductionen," under which are considered a series of marked phenomena observed in organisms of various grades of complexity, including *Planaria*, *Lumbricus*, *Æolosoma*, etc.

Similar experiments by Greeley ('03), on "Effects of Variations of Temperature on Animal Tissues," show essentially the same phenomena. Among these experiments some made on *Hydra* are especially pertinent in this connection. To quote, "It was at once observed that whenever a *Hydra* is exposed to a temperature of 4° to 6° C. the tentacles gradually become thicker and shorter, and finally are completely absorbed into the body. As the absorption goes on, the ectoderm and entoderm cells of the tentacles lose their individuality and form an undifferentiated mass of protoplasm, which is slowly resolved into the body of the *Hydra*. The tentacleless body of the *Hydra* becomes slowly resolved into a dense spherical mass of coagulated protoplasm, in which no distinction between individual cells can be made out, and remains in this condition as long as it is kept at a low temperature, but quickly forms tentacles and a double layer of cells again when it is returned to the temperature of the room" (p. 43).

Enough has now been said to show, I think, that only in some qualified sense can one use such terms as *irregular*, *meaningless*, *abnormal*, *pathological*, etc., in describing phenomena such as those involved in the experiments and results under review.

As a final note it may be stated that in my experiments no attempt was made to detach and isolate the several cell-aggregates such as was done by Wilson. Neither did I attempt to augment the masses by artificially bringing several masses into contact as he had done. Attention has already been directed to the fact that there was some evidence that from larger masses were derived larger polyps, and that below a certain minimum size there was no evidence of growth.

It need hardly be stated that my experiments add little essentially new to those of Wilson; yet they seem to afford valuable confirmation of some importance which may add to the conclusiveness of his admirably conceived and conducted research.

I desire also to express my gratification in the experiments of DeMorgan and Drew, which seem to me to have been admirably done and contribute to the value of the investigation as a whole.

SYRACUSE UNIVERSITY,
Jan. 20, 1915

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THE EYES OF CAMBARUS SETOSUS AND CAMBARUS PELLUCIDUS.[1]

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Cambarus setosus Faxon inhabits the caves of southwestern Missouri. It was described by Faxon in Garman's account of the "Cave Animals of Southwestern Missouri." An account of the eyes of this species was published by Parker.

In June, 1911, I secured an abundance of fresh material which made a reëxamination of the eyes desirable. I found it in the caves about Springfield, Sarcoxie and Ozark, Missouri. From Smallen's cave near Ozark I secured seventy-five specimens from 20 to 110 mm. in length. In addition to these, twenty-seven specimens of *C. pellucidus testii* Hay were taken from Mayfield's cave near Bloomington, Indiana. Also thirty-three *C. pellucidus* (Tellkampf) from Shawnee cave, Indiana University farm, near Mitchell, Indiana. The specimens from Indiana were collected during the fall and winter of 1911 and 1912. The blind crayfish from Indiana are smaller than those from Missouri. The smallest taken from Indiana caves were 9 mm. in length. Others varied from 15 to 60 mm. The largest specimen of *C. setosus* taken was 120 mm. in length.

C. setosus has been found only west of the Mississippi river, while *C. pellucidus* has been found only east of the Mississippi both north and south of the Ohio river. *C. pellucidus testii* Hay has been recorded only from Mayfield's Cave, Bloomington, Indiana. *C. pellucidus* has probably the widest distribution of any of the blind crayfish. It has been taken from Mammoth and other caves of Kentucky and from Wyandotte and other caves in Crawford county, from Clifty cave, Washington county, from Lost river, Orange county, from Shiloh, Down's, Donnehue's

[1] Contribution from the Zoölogical Laboratory of Indiana University, No. 146. This work has been carried on under the direction of Professor Carl H. Eigenmann, to whom I am thankful for advice and criticism.

and Donnelson's caves in Lawrence county, Indiana. Strong's, Truitt's, Kuntz's, Marengo, Little Wyandotte or Seibert's and several smaller caves of Indiana were examined for blind crayfish but none were found.

The caves of Indiana are in the same general geological forma-

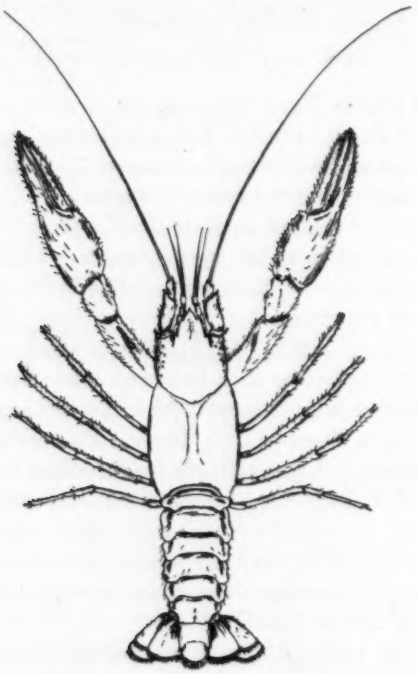
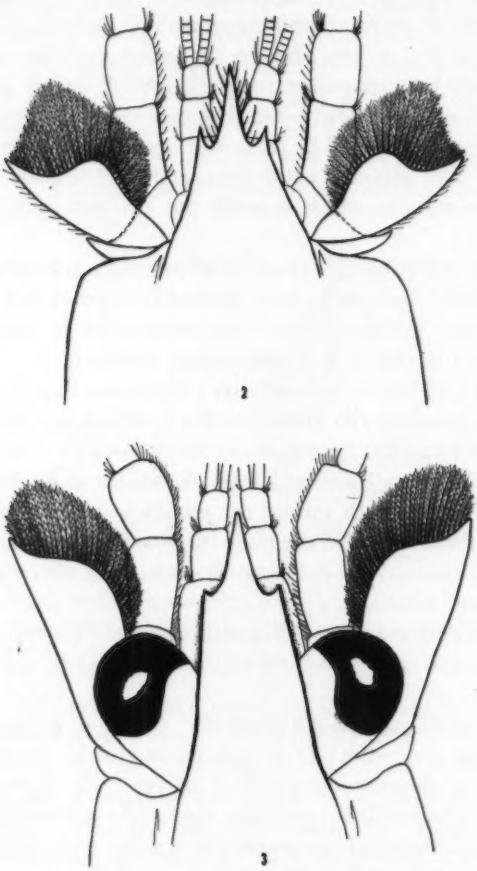


FIG. 1. Dorsal view of *C. setosus* 110 mm. long, with small, inconspicuous eyes extending slightly from under the rostrum. (Life size—reduced ½.) Drawn by Miss Dorothy L. VanDyke.

tion as those of Missouri. For an account of the Missouri caves see Shepard, in the Missouri Geological Survey, Vol. XII., 1898. For the fauna and geology of Indiana caves see Green, Indiana Academy of Science, 1908, and Blatchley, Indiana Department of Geology and Natural Resources, Twenty-first Annual Report, 1896.

The structure of the eyes in the blind crayfish, *C. pellucidus*, was first noted by Newport (1855). Concerning his specimens which were taken from Mammoth cave, Kentucky, he states:



FIGS. 2 AND 3. Dorsal views of the anterior ends of *C. setosus* and *C. propinquus*(?) 36 mm. long. They show the relative size and conspicuousness of the eyes of the two species.

"The hardened tegument which clothes the entire organ is thinnest and most transparent in that part of the eye which forms

the cornea in other Crustaceans; so that the eye may be unfitted for distinguishing form, the creature may yet possess the faculty of perceiving the small amount of actinic rays of light which might penetrate into its subterranean abode . . . ; the cornea also exhibits an appearance of being divided into a few imperfect corneals (facets) at the apex of the organ, and the struetures behind these into chambers, to which a small but distinct optic nerve is given." He also noted that the eyes are not pigmented.

I find that the eyes of *C. pellucidus* from Indiana caves and of *C. setosus* from Missouri caves show neither "corneals" (facets) nor "chambers, to which a small but distinct optic nerve is given."

The next writer on the eyes of blind crayfish was Leydig (1883). He stated that the cornea in *C. pellucidus* is lamellated, without pigment and without facets. His description of the internal structures of the eye is very general and indefinite.

Packard (1888) in his memoir on "The Cave Fauna of North America" describes and illustrates the form and structure of the eyes of *C. pellucidus* from Indiana and Kentucky caves and *C. hamulatus* Cope and Packard from Nickajack cave, Tennessee. He found that in both species the cornea is without facets and that the hypodermis is of the same thickness in the retinal region as in other parts of the eye; also that the optic nerve and optic ganglion are present.

The following year (1889) Garman published Faxon's description of *C. setosus* to which reference was made in the opening paragraph.

Parker (1890) published a paper on "The Eyes in Blind Crayfishes." He had access to *C. hamulatus* and *C. setosus*. The major part of his paper deals with *C. setosus*. He emphasizes the uniform thickness of the cuticula, the nearly uniform thickness of the hypodermis and the relation of the size and conical shape of the optic stalk to the amount of degeneration, as well as the histological structure of the degenerated eye. He also called attention to the relation of the axis of the cone, which is the terminal part of the optic stalk, to the axis of the stalk itself.

The most striking characteristics of the gross anatomy of the eyes of *C. setosus* and *C. pellucidus* are smallness, lack of pigmen-

tation and inconspicuousness. These features are shown in Figs. 1 and 2.

The eyes of all the blind crayfish examined are nearly covered by the rostrum, from a dorsal view, while the eyes of normal crayfish are only slightly concealed by the rostrum. However the eyes of the young blind crayfish are relatively larger than they are in the adult. The relative size of the eyes of *C. setosus* and *C. propinquus* (?) is shown in Figs. 2 and 3.

Size and pigmentation make the eyes of normal crayfish conspicuous. The eyes of blind crayfish are smaller and without

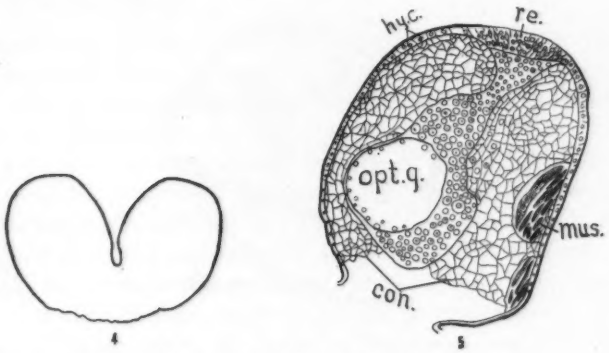


FIG. 4. Outline of the eyes of *C. pellucidus* 10 mm. long.

FIG. 5. Horizontal (longitudinal) section of the eye of *C. setosus*, with the cuticula removed.

pigment. The relative shortness of the optic stalk in the eyes of blind crayfish tends to make them inconspicuous.

The distal end of the optic stalk of the eyes of the blind crayfish examined is roundish or almost hemispherical in shape. Fig. 4 which shows this was made with the aid of a camera lucida, from a fresh specimen of *C. pellucidus*, 10 mm. long. The same general form of the eye is shown in photomicrographs *A* and *B*, which were made from a horizontal section of the eyes of *C. setosus*. In no case out of fifteen series of sections of the eyes of *C. setosus* and *C. pellucidus* have I found the exaggerated conical form figured by Packard and by Parker. Crayfish killed in Perenyi's fluid and kept in 85 per cent. alcohol for a few weeks

show considerable shrinkage of the connective tissue and after some months the cuticula shrinks. Dehydration and embedding also cause further shrinking. See photomicrographs *A* and *B*. The optic stalk may then approach the conical form observed by Packard and by Parker.

The cuticula of the eyes of *C. setosus* and *C. pellucidus* is usually smooth. Sometimes it is wrinkled by the fixer or preservative. These wrinkles may have led Newport to the conclusion that it is faceted. The main points of interest concerning the cuticula are; first, it is thinnest in that part of the optic stalk occupied by the cells of the vestigeal eye; second, it is laminated.

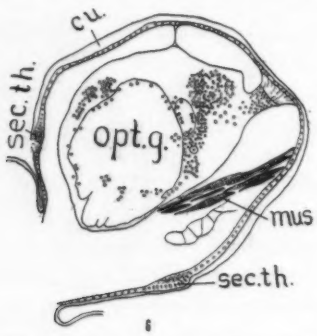


FIG. 6. Horizontal section of the eye of *C. setosus*, with the cuticula removed Made from a section near the one shown in photomicrograph *A*.

Sometimes the cuticula in *C. setosus* and in *C. pellucidus* is from two to three times as thick on the sides as it is on the anterior end or retinal region of the optic stalk. My photomicrographs *A* and *B* and Fig. 6 show that the cuticula is thinnest in the retinal region. According to Gilbert the average thickness of the retinal cuticula is 3.41 mm., while that of the sides of the stalk is 12.41 mm. This is quite contrary to Parker's statement that "The optic stalk is covered with a cuticula which is of uniform thickness."

It will be recalled that the cuticula is secreted by the hypodermis. The hypodermis which is of ectodermal origin becomes differentiated into the visual organ in Arthropoda. This differentiation consists of the thickening and invagination of the hypodermis.

The part of the degenerated eye of the blind crayfish which is of chief interest is the retinal hypodermis. Here the largest amount of degeneration has taken place. The optic ganglion, optic tract and brain show little or no signs of degeneration. The condition of these structures is well illustrated by photo-

FIG. 7. A secondary thickening of the hypodermis from the antero-median part of an eye of *C. setosus*.

micrographs *B*, *C* and *D*. The optic nerves (Fig. 8 and photomicrographs *C* and *E*) connecting the retinular cells with the optic ganglion are also present but they are not as well developed as the above mentioned structures.

It is probable that when the embryology of the eye of the blind crayfish is worked out, we may be able to definitely identify the retinal cells shown in Fig. 8. The eyes of a *C. setosus* 15 mm. long show about the same amount of degeneration as the eyes of adults. The arrangement and general appearance of the retinal cells of the hypodermis, shown in Fig. 8, is comparable to an early embryonic condition found in the developing eyes of many Crustacea, the bee and other Arthropoda.

Parker states that the hypodermis in *C. setosus* is "very nearly uniform in thickness." I find that the retinal hypodermis in *C. setosus* and *C. pellucidus* is quite irregular as to thickness. Sometimes there are as many as three and four different thickened regions in a single section. These thickened places in the hypodermis are found at various places around the anterior end and the sides of the optic stalk. The principal and most common thickening is in the anterio-lateral part of the optic stalk, as is shown in Fig. 5 and photomicrographs *B*, *C* and *E*. Secondary thickenings are sometimes low on the sides of the optic stalk, as illustrated by Fig. 6 and photomicrograph *A*. Fig. 7 represents a type of the secondary thickenings. It was taken from a portion of the hypodermis along the anterio-median part of the right eye of *C. setosus*. The most highly developed or the least

degenerated of these thickenings are in the anterio-lateral or retinal portion of the optic stalk. This point is well illustrated in Figs. 5 and 8 and photomicrographs *B*, *C* and *E*.

The retinal region of the hypodermis has two or three distinct layers of cells, while the rest of the hypodermis has only one layer of cells, except in secondary thickenings such as are shown in Figs. 6 and 7 and photomicrograph *A*. The cells of the retinal hypodermis in *C. setosus* and *C. pellucidus* differ in size, shape and staining properties from the other cells of the hypodermis. My drawings and photomicrographs show that the hypodermis is considerable thicker in the retinal region than elsewhere. Here

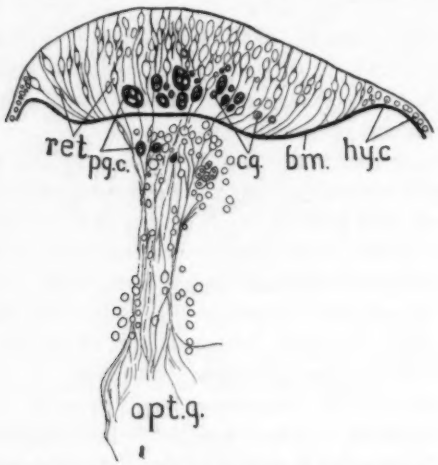


FIG. 8. Cell structure of the retinal region of the eye of *C. setosus* from a section similar to those of photomicrographs *B*, *C* and *E*.

again I differ from Parker who said of the hypodermis in *C. setosus:* "At least it is not thicker in the region of the retina than at many other places."

The ommatidium or ocellus (Fig. 9) is the unit of structure of the compound eyes of Arthropoda. The functional eye of *Cambarus* has four layers of cells in the developing retinal hypodermis; the corneagen, the vitrellæ, the retinulæ and pigment cells.

The diopteric structures of the eye, such as the lens, cone and

rhabdom are absent in the eyes of *C. setosus* and *C. pellucidus*. Some of the sensory cells are present even though they may be no longer functional. I believe we may feel reasonably sure of the identity of some of the cells found in these degenerated eyes.

In Fig. 8 there are at least three kinds of cells shown; (1) the large, oval, dark staining, granular cells with several nuclei, the "granular bodies," "degenerated representatives of the cones in the normal eyes" of Parker, (2) elongated cells or nuclei with fibers, and (3) small, round, granular cells with clear nuclei.

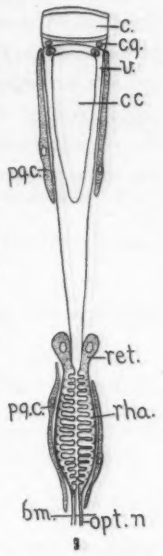


FIG. 9. The ommatidium of *Cambarus*. (After Watase.)

The large, oval, dark staining, granular cells with several nuclei are probably pigment cells as they are the only similar cells found on both sides of the basement membrane in the functional eyes as well as in these degenerated ones. Parker found "granular bodies" on the distal side only of the basement membrane and called them "degenerated representatives of the cones in the normal eye."

The elongated cells with fibers compare with the retinulæ of the functional eye. The retinulæ are the only cells with fibers in retina.

The small, round, granular cells with clear nuclei may be degenerated cone cells or the "undifferentiated hypodermal cells" of Parker. But most of these cells are too far removed from the cuticula to be "undifferentiated hypodermal cells" which have secreted the cuticula. The cuticula is thin in the retinal region. According to Watase, the sensory cells of the ommatidium secrete the cuticula. I am inclined to believe that the small, round, granular cells are degenerated cone cells.

Comparing the sections of the eyes of *C. setosus* and of *C. pellucidus* it is found that the eyes of *setosus* are little if any more degenerated than those of *pellucidus*. Irregularities are common in the retinal hypodermis of the eyes of these crayfish.

Whether the eyes of the blind crayfish have passed through a higher stage of development and then degenerated or whether

their development has been arrested at this stage, can be determined only by a study of the developing eyes.

However I believe that the degenerated eyes of *C. setosus* and *C. pellucidus* are instances of arrested development rather than examples of degeneration. The reasons for this belief are that the eyes as found in the adult have a cell structure which appears to be comparable to the developing eyes of Crustacea. The radiate arrangement of the retinal cells of the hypodermis, is suggestive of the developing ommatidium. Also the eyes of the young blind crayfish, *C. setosus* and *C. pellucidus*, show about the same amount of degeneration as the eyes of the adult.

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EXPLANATION OF FIGURES AND ABBREVIATIONS.

The tissue was fixed with Perenyi's fluid. The sections from which the drawings and photomicrographs were made were cut eight microns in thickness and stained with Haidenhain's iron hæmatoxylin. The drawings were made with the aid of a camera lucida.

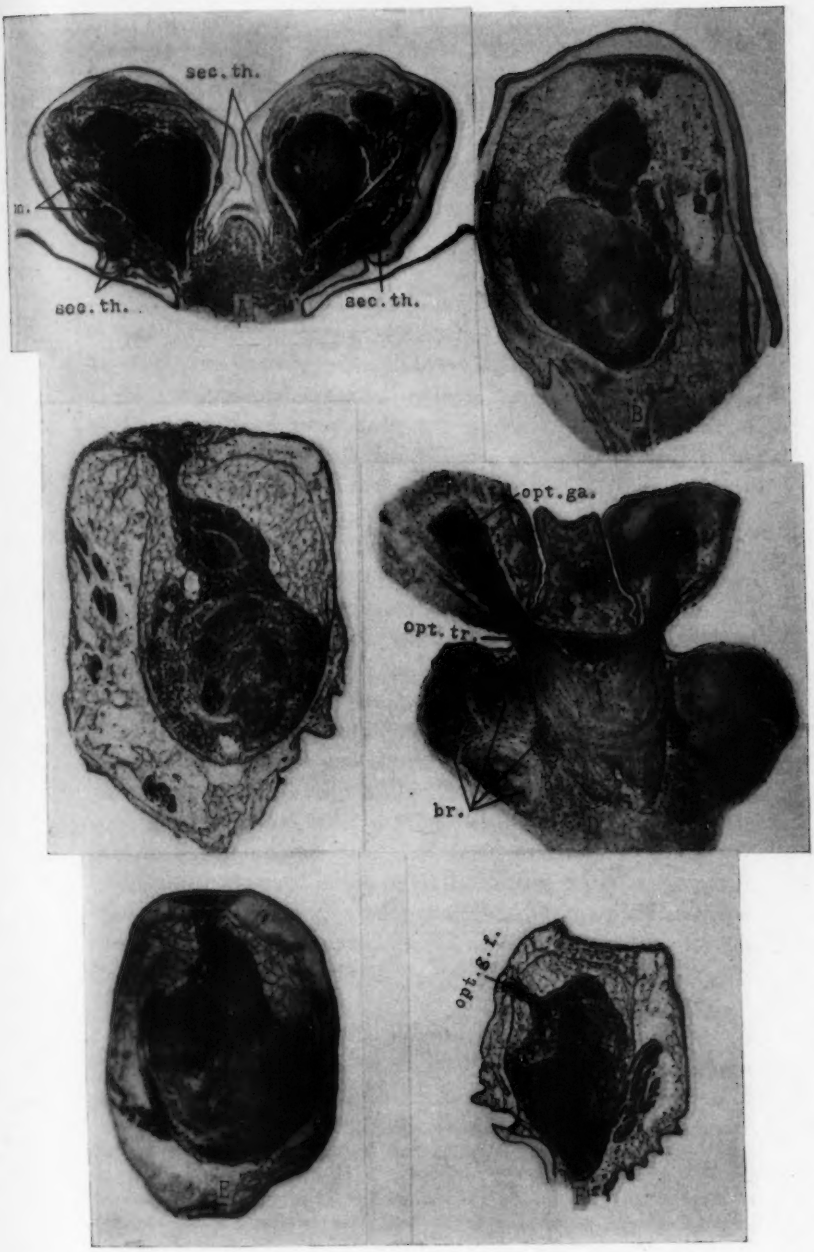
bm., basement membrane.
br., brain.
c., cornea.
cc, crystalline cone.
cg., corneagen cell.
con., connective tissue.
cu., cuticula.
hy. c., hypodermal cells.
mus., muscle.
opt. g., optic ganglion.
opt. g. f., optic ganglion fibers.
opt. n., optic nerve.
opt. tr., optic tract.
pg. c., pigment cell.
re., retina.
ret., retinulæ.
rha., rhabdom.
sec. th., secondary thickenings of the hypodermis.
v., vitrella.

PHOTOMICROGRAPH *A*. Horizontal section of the eyes of *C. setosus*.

PHOTOMICROGRAPHS *B* AND *C*. Horizontal sections of the eyes of *C. setosus*. *C* has the cuticula removed. The cuticula in *B* is thinner in the retinal than it is on the sides of the optic stalk. It is not as thick as it appears to be in the retinal region, due to the fact that it is wrinkled. It is also pulled loose from the underlying hypodermis, caused by the shrinking of the more delicate tissues.

PHOTOMICROGRAPH *D*. Horizontal section of the brain of *C. setosus*.

PHOTOMICROGRAPHS *E* AND *F*. Horizontal sections of the eyes of *C. pellucidus*. The cuticula has been removed. *F* has a tract of nerve fibers extending anteriomedianly towards the hypodermis, but there is no thickened portion of the hypodermis with which it is connected.



CHARLES H. SPURGEON.

STUDIES ON TISSUES OF FASTING ANIMALS.

S. MORGULIS, PAUL E. HOWE AND P. B. HAWK.

The changes rendered in the finer structure of tissues of fasting animals have been extensively studied and the results of these investigations have an important bearing upon our understanding of the inanition phenomena in general. Apart from the interest which the subject presents from a purely histological point of view, it throws light on many obscure problems regarding the transformation of materials within the organism occasioned by the fast.

The account here presented is based upon an examination of tissues from several dogs and one fox which had died of protracted inanition, having previously suffered a very large loss in body weight. These animals had been used in a number of metabolism experiments[1] conducted some years ago in the University of Illinois.[2] The tissues were removed immediately after the animal's death and fixed in Teleschnitzky's and Zenker's fluids. The material was carried through graduated alcohols and then preserved in eighty per cent. alcohol. It was embedded in paraffin, sectioned and stained in Delafield's hæmotoxylin, with eosin as a counterstain.

A superficial examination of the sectioned material, except in a few instances, reveals nothing abnormal. But a little attentive study is sufficient to appreciate the different ways in which the effect of prolonged inanition is stamped upon the histological elements of the organism.

Looking at the smooth muscles in the intestinal tract of every one of the animals which died of fasting the cells appear turbid and without a trace of longitudinal fibrillation. The

[1] Howe, Mattill and Hawk, *Jour. Biol. Chem.*, 10, 417, 1911 and 11, 103, 1912. Howe and Hawk, *Jour. Am. Chem. Soc.*, 33, 215, 1911; *Am. Jour. of Physiology*, 29, xiv., 1912, and 30, 174, 1912.

[2] We take this opportunity to acknowledge the material assistance which we received from the department of chemistry of the University of Illinois in defraying the expenses of the research.

fibers seem widely separated from each other, giving the entire muscle a very loose appearance. In cross section they are seen to consist of a dense central portion, which stains more or less strongly, surrounded by a colorless material. There is, however, no indication of a swelling of the muscle fibers as there is likewise no evidence of fatty degeneration, but they apparently undergo a process of liquefaction similar to that described by Miescher in the Rhein Salmon occasioned also by protracted fasting while it remains in fresh water. The nuclei are extremely irregular in outline and stain faintly.

In the voluntary, or striated muscle fibers, the cross markings lack the usual distinctness. Swelling or granular degeneration, such as described by Statkewitch, was never seen in our material.

Of all the organs of the body the liver is taxed most heavily during inanition inasmuch as it must take care of the products of metabolic activity of all other organs besides sustaining itself. It is natural to expect, therefore, to find the changes in the structure of the liver cells of a most pronounced character. Indeed, in the material under our examination a variety of degenerative phenomena has been observed. Considering the great difference in the degree of degeneration of the liver from animals which have all died of starvation it follows that death is not necessarily preceded by extreme cellular transformation. In our material every gradation from very slight changes to complete fatty degeneration of the liver cells could be observed. In two dogs which fasted 30 and 48 days respectively, whereby they lost 46 and 53 per cent. of their weight, there has been very little fatty degeneration in the liver. Some cells, however, were coarsely granular and others were riddled with vacuoles. In the case of the fox, which in 13 days of absolute fasting lost only 13 per cent. of its weight, the cells were found to be hollowed out by vacuoles of various sizes. These frequently encroach upon the nucleus and distort its shape as may be seen in Fig. 1. The vacuoles never show a very sharp outline, their boundary being more commonly diffuse and indefinite. In one extreme case of degeneration the liver presented complete transformation of its cells into typical fat cells. The polygonal shape of the cells was retained but the protoplasm was reduced to a mere band enclosing

a mass of fat. The cells seemed rather distended. The nuclei, pushed out to the periphery and usually into a recess of a corner, were flattened against the wall. Their staining capacity as well as that of the protoplasm was very feeble. The fatty degeneration was not equally intense in every portion of the liver, and here and there groups of intact liver cells could be seen whose poor staining power was the only evidence of degeneration.

No particular changes have been observed in nuclei. Cells with more than one nucleus are not uncommon, but these are found also in the normal liver. Phenomena of chromatolysis and vacuolization of the nuclei described by Statkewitch were never observed by us.

The histological structure of the stomach and intestine shows no striking changes. In sections of the stomach the oxyntic or parietal cells of the fundus glands are most conspicuous owing to their relatively large size and deep staining capacity. Their protoplasm is very granular. The other cells of the gland are small and their protoplasm is thin and practically colorless. The nuclei are usually normal, but in some portions, especially near the proximal end of the gland, they are much elongated and pressed against the cell wall adjoining the basal membrane. The two figures in the plate, 2 and 3, one a cross section of the upper region of the gland, the other a longitudinal section through the base of the gland, show these points. The clear, transparent character of the protoplasm is very well seen in the former, Fig. 2. The nuclei are always near the basal membrane.

The points brought out in the study of the fundus glands are also essential for all other glands as well as the mucous membrane of the intestine. The cells stain very feebly, their protoplasm being free of any granules. The nuclei migrate toward the basal membrane.

The phenomenon of particular interest, especially when viewed in the light of certain results of bacteriological studies on the permeability of the intestinal canal, is the invasion of the tissue underlying the mucous membrane as well as of the cells of the mucous membrane itself by numerous leucocytes. These occur not only singly but in groups of several cells together and occasionally accumulate in masses resembling solitary glands.

The submaxillary gland presents a few changes which are worth pointing out. The protoplasm of its cells, as was seen also in other gland cells, is thin in character and fails to take up the stain. Many cells are without nuclei, and the darkly stained crescent cells though present are generally flattened out and cap the outside of the alveoli like a narrow band. Fig. 4 shows that the submaxillary gland at the fatal termination of a protracted fast has all the appearance of a resting gland.

The most interesting set of modifications is to be observed in the kidneys. There one encounters various forms of degeneration and their distribution in the kidney is quite significant. We have already mentioned in discussing the changes in the liver that the extent of the degeneration of the histological elements apparently bears no relation to the death of the fasting animal as one of its direct causes. This statement holds equally true for the kidneys, where we found likewise a very wide range of modifications at the time of the animal's death.

The glomerulus has the usual lobulated structure but the Bowman capsule enclosing it is invariably thickened as in the case of nephritic kidneys. The cells of the convoluted tubules have a coarsely granular content and are invariably vacuolated. In some instances the vacuolization is so extensive as to give the tubule a striking honeycombed appearance. In Fig. 5 which is from a section of the kidney of the fox, this is shown very clearly. Similarly Fig. 6, which represents a section of a tubule in the kidney of a fasting dog, shows extensive vacuolization and the absence of boundaries between the cells. This last phenomenon, namely, the formation of a syncitium is characteristic not only of the kidney but also of the liver where the cells seem to melt together. In the ascending and descending limb of Henle's loop, however, vacuolization is a very rare occurrence, particularly in the later. The tubules were generally very granular in structure and contained frequently casts of various kinds, cellular, hyaline, etc. Fig. 5 is interesting furthermore on account of the well-preserved ciliated band lining the lumen of the tubule. The nuclei of the tubules are small and more or less irregular in shape. The cells of the collecting tubules show hardly any effect. The protoplasmic content is very clear

and free of granules. Here and there cells are found which have no nuclei. But when present the nuclei are relatively large and round, frequently bulging out into the lumen owing to the diminution of the cubical cells.

Before concluding this description of the changes which were observed in the tissues of fasting animals, a few words may be said concerning the condition of the testes and ovaries. In the former we failed to find any dividing cells. The nuclei were of the characteristic large round shape, whose chromatic content was intensely stained. It is noteworthy that in a very large proportion of the tubules the chromatic substance of all the nuclei was massed together to one side, as in the case of synizesis. It is hardly possible that this should be due to an artifact, as the nuclear condition varied in different tubules, but the former was found in most of them. The ovary which was examined seemed normal in every respect with numerous eggs in all stages of growth. We examined a number of fully developed eggs which were perfectly normal in every detail of their structure.

Bearing in mind that in inanition the organism is obliged to draw upon its own resources to derive the energy necessary for its maintenance, the metaplasmic material stored up in its cells and the depôts of fat are first to yield their quota to this stringent need. With the prolongation of the fast, as these reserve materials become reduced in quantity and at last disappear altogether, the substance of the cell body proper must contribute to the organism's demand for nourishment. It is now a well-established fact that various organs and tissues share unevenly in the support of the starving organism. As would be expected *a priori*, those elements of the organism the integrity of which is indispensable to its continued existence resist the pressure of the unfavorable conditions longest. This is true not only for the different systems of organs, but also for the minutest element of the organism, the cell, where the nucleus is usually the last part to fall prey to the exhausting effect of the fast. The nervous system likewise maintains its weight practically at a constant level as well as it preserves its morphological integrity until a very advanced stage in the fast.

Degenerative changes do not, as a rule, occur in any of the

tissues so long as the reserves of the body have not yet been entirely exhausted. The early appearance of fat globules in the liver of fasting animals led Mottram to believe that this process must be a physiological and not a pathological one. A similar opinion was likewise expressed earlier by Gilbert et Jomier. Mottram showed by means of histological examination of the liver of rabbits and guinea-pigs as well as by actual chemical investigation that with the advance of the fast an infiltration of the liver cells with fat from the depôts does take place. None of the authors who gave attention to this matter studied the liver of animals in very advanced stages of a fast. In our own case the animals succumbed after a loss of about 50 per cent. of their weight. There was very little histological evidence of a fat accumulation, but vacuolization of the cells was most prominent. It is hardly conceivable that the vacuoles were produced by the removal of the fat content by the reagents used in preservation in as much as it has been shown above that in one instance a liver was observed the cells of which in certain localities have undergone complete fatty degeneration. We are aware of the fact, of course, that a parallelism does not exist between histologically and chemically demonstrable fat in tissues. It may be that the infiltration of the liver with fat, especially in the early period of fasting, which is now proven beyond reasonable doubt (Mottram, Smirnow) and is very properly considered a physiological phenomenon, is concerned with the transfer of depôt fat to the rest of the tissues as food, while fatty degeneration of the liver cells, such as we observed over certain areas, is an independent phenomenon and is accompanied by the loss of the normal functional power of the cells. The view expressed here that the infiltration of the liver with fat may have to do with the conveying of the fat as nutriment to the starving tissues is borne out by Mottram's interesting observation on the qualitative change of the liver fat on different days of a short fast. There is a striking parallelism between the pure fatty acids present in the liver and the fat-quotient, *i. e.*, the ratio of the total fat of the liver to the initial body weight, showing that whenever an increase in fat content, *i. e.*, an infiltration, occurs it is due to an accumulation of fatty acids. If this view of the

rôle of the liver is correct it may also clear up the problem of the cause of the premortal rise in the nitrogen elimination. The latter was thought to be due either to an exhaustion of the entire supply of fat or to an excessive disintegration of cells. Neither the one nor the other of these hypotheses can be considered beyond criticism, because even in animals succumbing to a much protracted fast there is still sufficient fat present,[1] whereas there is no histological evidence of an unusual cellular destruction towards the end of the fast. A morphological and physiological degeneration of the liver interfering in some manner unknown to us with the endogenous fat metabolism probably results in an increased demand upon the body proteins which hastens the death of the animal.

In this connection it is interesting to point out that fat has never been demonstrated in tissues which in the fasting organism are among the strongest consumers, such as nervous and muscular tissues. The glandular tissues on the other hand, which are more or less deprived of their proper activity during a fast invariably show the presence of fat globules, according to Nicolaides. Later these fat globules disappear, leaving "empty spaces" which evidently correspond to our vacuoles. Nicolaides observed that in the gland cells of the duodenum and the pylorus small fat globules appear as soon as the animals commence to fast, whereby they invariably assume a regular arrangement in two parallel rows. We cannot agree with Nicolaides who considers the fat globules as "degenerative," and certainly see no reason for his assumption,—since their arrangement within the cell points against the supposition of a migration from fat depôts,—that they are formed from the protein constituents. The fact that in the submaxillary gland the fat globules appear only in the albuminous cells but never in the mucous or crescent cells, which has been observed by both Statkewitch and Nicolaides, cannot be taken as good proof of a formation of fat from protein. Also these facts become plain in the light of our hypothesis that the early appearance of the fat globules speaks decisively against any supposition that they result from degenerative transformation. We believe, on the contrary, that this is

[1] In the case of a dog which fasted 117 days and subsequently 104 days there were large masses of fatty tissue in the abdominal cavity at the time of death.

simply due to a qualitative transformation of the fat present in the cells whereby it becomes histologically visible. Since the staining reagents which are used in demonstrating the presence of fat globules are such that they react only with unsaturated fats, the appearance of the globules indicates that with the beginning of the fast a process of desaturation and probably the formation of fatty acids is started preliminary to the absorption of this fat to serve the nutritional needs of the organism.

The results of studies on fasting unicellular organisms where the conditions are simpler and easier to be appreciated support the view that vacuolization is one of the earliest and the most common degenerative process which ensues with the exhaustion of the reserves of the cell. Wallengren indeed in his most valuable research on inanition of infusoria distinguishes two periods, before and after the reserve material is exhausted. The former is accompanied by a gradual diminution of the animal, while in the second period the endoplasm becomes honey-combed with vacuoles of various sizes.

Vacuolization has been observed in various tissues: in ganglionic cells of the heart (Statkewitch), in the cells of the motor ganglia of the anterior horn (Schäffer) in bone marrow (Soltz), in the nephridial epithelium, etc.

Before concluding the paper mention should be made of another degenerative process which develops in the course of inanition. We refer to the gradual melting away of the cell boundaries which, with their complete disappearance, may even result in the formation of a syncytium. One of us described this condition in the liver of fasting salamanders. We also observed this phenomenon in our liver and kidney preparations. Similar observations have been made in fasting lower animals (Schultz). In the study of the salamanders it was shown how rapidly the cell walls are built up again around the intact nuclei as soon as the emaciated animals are once more given food.

At last the more or less universal loss of staining capacity due to the degenerative transformation produced by inanition and described by practically all authors must be pointed out.

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EXPLANATION OF FIGURES.

All figures have been made with the camera lucida and under the same magnification, using an objective No. 6 with an ocular piece No. 4 at normal tube length.

FIG. 1. A group of liver cells of a fasting fox.

FIGS. 2 AND 3. Cross-section and longitudinal section of fundus gland of a fasting dog.

FIG. 4. Section of submaxillary gland of fasting dog.

FIG. 5. Section of tubules in kidney of fasting fox.

FIGS. 6 AND 7. Section of convoluted tubule and of Henle's loop in kidney of fasting dog.

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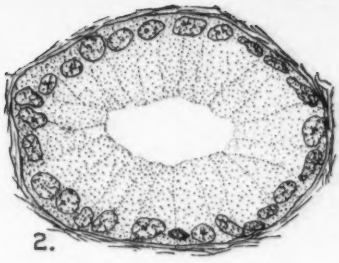

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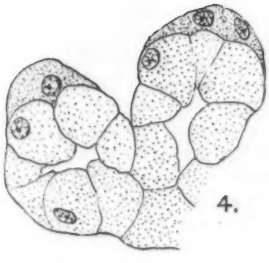

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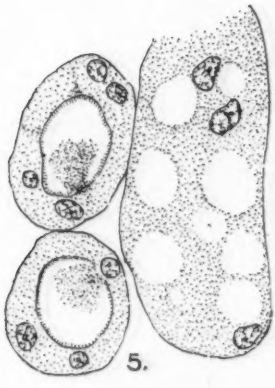

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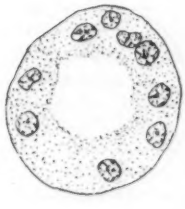

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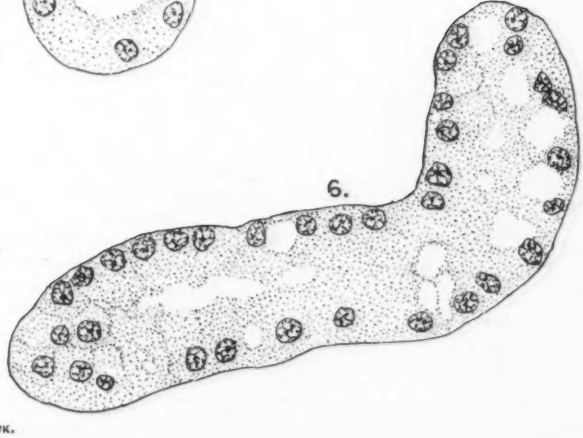

6.

THE OLFACTORY SENSE OF COLEOPTERA

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INTRODUCTION AND METHODS.

In the investigation here recorded two objects have been kept in view: (1) To make a careful study of the morphology and physiology of the olfactory pores of beetles, and (2) to determine experimentally whether or not the olfactory organs lie in the antennæ.

Since those investigators, who have performed experiments on beetles with mutilated antennæ, have failed to study sufficiently the behavior of the insects investigated, the responses observed have misled them in determining the seat of the olfactory organs. Entomologists are generally agreed that the organs of smell in beetles lie in the antennæ, but when the results of those who have performed experiments on beetles are carefully considered, it is seen that some beetles with amputated antennæ smell practically as well as unmutilated ones, while other beetles are materially affected when the antennæ are mutilated. Hicks (1857 and 1860) discovered some peculiar organs (called olfactory pores by the present writer) on the wings and legs of beetles and he suggested that they have an olfactory function. Lehr[1] discovered the same organs on the peduncles of the elytra of *Dytiscus marginalis*. The present writer ('14*a* and *b*) made a comprehensive study of the olfactory pores in Hymenoptera and he ('14*c*) gives a complete review of the literature pertaining to the sense of smell in insects. The present paper embodies the results of a careful study of the olfactory pores in Coleoptera in much the same manner as pursued on those in Hymenoptera.

To obtain material for the study of the disposition of the olfactory pores, adult specimens were used. In regard to prepar-

[1] Lehr's paper, which deals only with the morphology of these organs, was overlooked until after my paper had been sent to press. Lehr has not seen any of my papers on this subject because my first one ('14*a*) appeared only three months before his, and my second one ('14*b*) appeared in the same month as his.

ing the specimens with caustic potash and to bleaching them with chlorine gas, the reader is referred to the writer's work on Hymenoptera ('14*b*, p. 295).

To obtain material for the study of the internal anatomy of the organs herein discussed, beetles just emerging from the last pupal stage were mostly used. At this stage the chitin is soft, the wings are usually expanded, and the sense organs are fully developed. In order that the desired stages of beetles might be had, many larvæ and pupæ of various Coleoptera were collected on plants and in rotten stumps and logs. These immature insects were reared in the laboratory. When each one of them had reached the proper stage, it was killed and parts of it were put into a fixing fluid.

The writer ('14*a*, p. 268) describes the usual method of embedding with celloidin and paraffin. Since then, a rapid method has been used which is described in detail as follows: The various appendages of the insects are removed, and are cut into small pieces, which are immediately dropped into a modification of Carnoy's fixing fluid. This fluid, containing equal parts of absolute alcohol, chloroform, and glacial acetic acid, with corrosive sublimate to excess, should be kept in a glass-stoppered bottle so that it may not lose its fixing ability by air being mixed with it. Also, while dropping material into vials containing this fluid, the stoppers of the vials should not be removed longer than absolutely necessary. When the material sinks to the bottom of the vial, it is removed and is thoroughly washed in 85 per cent. alcohol. It is then preserved in 85 per cent. alcohol. When ready for embedding, the material is cut into pieces from two to four millimeters in length. These pieces are then put into 95 per cent. alcohol containing eosin. When sufficiently stained, they are placed in a vial containing absolute alcohol and cedar oil. As soon as they sink through the alcohol into the oil and lie on the bottom of the vial, the alcohol and oil are removed. A small amount of ether is then poured into the vial. Five minutes later the ether is removed, and thin celloidin is poured into the vial. Ten minutes still later the thin celloidin is exchanged for thick celloidin. After remaining in the thick celloidin five minutes, the pieces of material are removed and are put into a vial of

chloroform where they remain five minutes. They are then embedded in 55° M.P. paraffin for five minutes. The sections were cut from five to ten microns in thickness and when they failed to ribbon the microtome knife was warmed. From this stage on the sections are treated like ordinary paraffin sections with the following exceptions. A rather thick film of fresh Mayer's albumen is spread upon each slide. After drawing the water from the slide upon which are mounted the sections, the latter are flattened to the slide by using a piece of wet tissue paper. No heat is used for straightening the ribbons on the slides because the least amount of heat blisters the celloidin. After drying over night, most of the sections adhere to the slides while being passed through the reagents, but to be sure of not losing any sections, the slides were sometimes wrapped in tissue paper and thread was then firmly wound around the paper. Instead of using absolute alcohol a mixture of equal parts of absolute alcohol and chloroform is employed so that the celloidin may not be dissolved, and instead of using eosin in 95 per cent. alcohol as a counter stain, the eosin is put into a mixture of the absolute alcohol and chloroform. The sections were stained in Ehrlich's hematoxylin from 10 to 15 minutes, the time depending on their thickness and whether or not they were wrapped in tissue paper.

The writer is grateful to Mr. H. S. Barber of the Bureau of Entomology for most of the dried specimens used which belonged to the collections of the U. S. National Museum. Mr. Barber is also to be thanked for the identification of all the beetles used in the experimental part of this work.

MORPHOLOGY OF THE OLFACTORY PORES.

Before experimenting to determine the function of the organs called the olfactory pores by the writer ('14*a*), the distribution and number of these pores in many beetles were studied.

Disposition.

In making a comparative study of the disposition of the olfactory pores in beetles, 50 species, belonging to 47 genera and representing 34 families, were used. With the exception of two

species used for individual and sexual variations, only one specimen of each species was studied. Whenever a portion of an appendage or an entire appendage was missing or was badly mutilated in being prepared for study, the number of pores on this portion or entire appendage was regarded the same as the number found on the corresponding portion or entire appendage on the opposite side of the body. Since the pores on only one specimen for each species were counted, the total number of pores recorded can not be a fair average. Besides this error, there is also a probable error of not less than 10 per cent. on an average for all the specimens. In the smaller specimens the probable error is perhaps not more than two or three per cent., but in some of the larger ones, this error is probably more than 10 per cent. The pores on only the legs, elytra and wings are included in the total numbers. Other parts of the insects were not examined, and it is quite possible that olfactory pores may be found on some of the parts not examined, particularly on the mouth parts.

(*a*) *Epilachna borealis*.

Since the lady beetle, *Epilachna borealis*, is most conveniently studied and as its pores are typical for most of the smaller beetles, the disposition of its pores will be described in detail, and then the variations found in the other species will be given.

The elytra and wings have dorsal and ventral surfaces, and the legs may be divided for description into two surfaces. The inner surface faces the body of the beetle and the outer surface is directed from the body. On the specimen examined, one group of pores was found on the peduncle of each elytron; three groups besides a few scattered pores on each wing; and two groups besides a few scattered pores on each leg. The groups and scattered pores are located as follows: Group No. 1 lies on the dorsal surface of the peduncle of the elytron with its distal or broader end against or just beneath the basal margin of the elytron (Text-fig. 1*A*, *BM*). Under a high-power lens, it is seen that this group lies on the radial plate (Text-fig. 1*B*, *RP*) between the muscle disk (*MD*) and the subcostal head (*ScH*). The distal ends of these heavy chitinous plates sometimes lie beneath the

basal margin (*BM*) of the elytron so that all or a portion of the group may be concealed. In such a case it is necessary to pull the peduncle from beneath the base of the elytron in order to count the pores. Group No. 1 on the left elytron consists of 71 pores (Plate I., Fig. 4), while the same group on the right elytron has 78 pores.

Groups Nos. 2, 3 and 4 lie on the dorsal surface of the wing on the radius (Text-fig. 1*C*, *R*). No. 2 lies on the extreme anterior

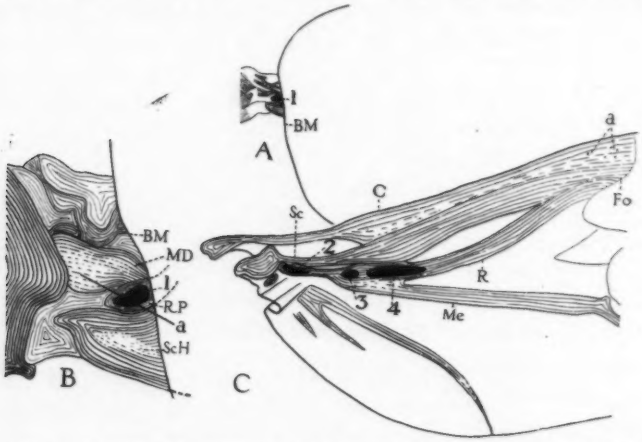

FIG. 1. Portion of left elytron and left wing of the lady beetle, *Epilachna borealis*, showing groups 1 to 4 of olfactory pores, as indicated by the numbers 1 to 4; *A* shows relative sizes of peduncle of elytron and group of pores on peduncle when compared with size of basal margin (*BM*) of elytron; *A* and *B*, dorsal surface of peduncle of elytron, showing position of group 1 of olfactory pores on radial plate (*RP*) between muscle disk (*MD*) and subcostal head (*ScH*). The lower side of each drawing is the outer margin of the elytron. *A*, × 8; *B*, × 45; *C*, dorsal surface of wing, showing position of groups 2 to 4 of olfactory pores on radius (*R*), × 8; *a*, position of scattered pores on ventral side of wing on union of costa (*C*) and subcosta (*Sc*) near fold of wing (*Fo*). Sometimes a group is found on the media (*Me*) just below group 4.

end of the radius and it is usually difficult to count its pores, because the surface of the radius at this place is greatly arched causing some of the pores to lie on the top of the arch while the remainder of them lie on the side of the arch facing the anterior margin of the wing. Nos. 3 and 4 are found on the radius where the media (*Me*) joins the radius. On the right wing, No. 2

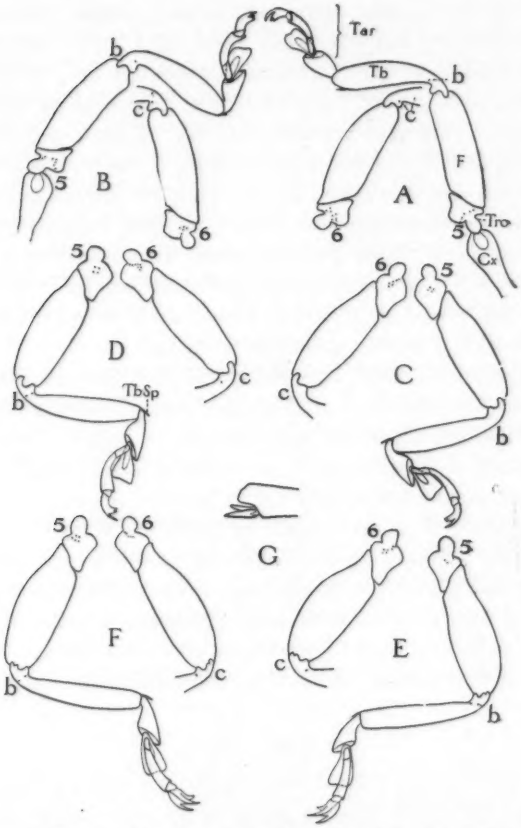

FIG. 2. Position of olfactory pores on legs of beetles, × 8; *A–F*, legs of lady beetle, *Epilachna borealis*, showing position of groups 5, 6, *b* and *c* of olfactory pores. The drawing of each leg in which the tarsus (*Tar*) is shown represents the outer surface of that leg, and the drawing not showing the tarsus represents the inner surface of the same leg. *A*, right front leg; *B*, left front leg; *C*, right middle leg; *D*, left middle leg; *E*, right hind leg; *F*, left hind leg. *G*, distal end of tibia from front leg of *Epicauta marginata*, showing five olfactory pores on one of the two tibial spines.

consists of 55 pores; No. 3 of 43 pores; and No. 4 of 43 pores. On the left wing, No. 2 consists of 50 pores; No. 3 of 43 pores; and No. 4 of 46 pores.

Group *a* of the scattered pores lies on the ventral surface of the

wing near the anterior margin of the wing a short distance from the place where the wing folds (Text-fig. 1*C*, *a*). On the right wing it consists of three pores and on the left wing of five pores.

Groups Nos. 5 and 6 are located at the proximal end of the trochanter (Text-fig. 2*A–F*, *Tro*), No. 5 lying on the outer surface and No. 6 on the inner surface. No. 5 usually extends only about half way across the leg, while No. 6 extends nearly all the distance across the leg. No. 5 on each leg consists of five pores, except on the left front leg where there are seven in it. On the right side No. 6 on each leg consists of seven pores, whereas on the left side on each of the middle and hind legs it consists of eight pores but of only six on the front leg.

Groups *b* and *c* of the scattered pores lie at the proximal end of the tibia (Text-fig. 2*A–F*, *Tb*), group *b* being located on the outer surface and group *c* on the inner surface. Group *b* on each front leg consists of only one pore; on the right middle leg it has one pore, but on the left middle leg it has two pores; on the right hind leg it has three pores while on the left hind leg it consists of two pores. Group *c* on each front leg has three pores, whereas on each of the middle and hind legs it has only one pore.

All six legs of the specimen of *Epilachna borealis* examined bear 95 olfactory pores; both elytra carry 149 pores, and both wings carry 288 pores. All of these combined make 532 olfactory pores.

(*b*) *Other Species.*

The greatest variation found in the olfactory pores of the other species examined is in regard to the total numbers of the pores. The second greatest variation is in regard to the distribution of the pores on the wings. This variation and other minor ones will now be given and a discussion of the total numbers of the pores will be presented last. For sake of brevity, instead of using the long scientific names of the beetles, the species will be numbered from 1 to 50, and those interested in associating the names of the species with the variations described may do so by referring to the names and numbers of the species in the table on page 419.

A group of pores (No. 1) was found on the peduncle of each elytron. This group in 46 species is definite, that is, the pores

are close together and are not scattered as they are in the other four species (Nos. 2, 34, 35, 44). In some beetles it is almost impossible to identify the various chitinous plates in the peduncles of the elytra, but as far as can be ascertained the definite groups of pores are located on the radial plates, while the scattered groups may spread over two or more of the plates. In shape these groups are round, oblong and triangular. The triangular-shaped ones are most common. As a rule, the more pores in this group, the smaller they are and the closer they are together. In three species (Nos. 23, 29, 36) the pores in this group are comparatively large, while those in the lady beetles are medium in size. *Osmus* with 12 pores on both elytra has the least number and *Hydrophilus* with 310 pores on both elytra has the largest number. In regard to the total numbers of pores on the elytra for the 50 species, the reader is referred to the table on page 419.

The three beetles, *Osmus*, *Clinidium* and *Cysteodemus*, are wingless. No rudiments of the wings were even found. The number of groups of pores on each wing of the other species varies from 1 to 4. Ten species (Nos. 1, 10, 13, 14, 16, 17, 18, 21, 22, 47) have only one group on each wing. One wing of *Lucidota* has one group while the other wing has two groups. Twenty-one species (Nos. 4, 5, 8, 11, 20, 24, 25, 26, 28, 30, 31, 32, 36, 38, 40, 42, 43, 45, 46, 49, 50) have two groups on each wing. Twelve species (Nos. 3, 6, 7, 9, 15, 23, 29, 33, 37, 39, 41, 48) have three groups on each wing. Three species (Nos. 12, 34, 35) have four groups on each wing. When only one group is present on each wing it usually occupies the position of Nos. 3 and 4 of *Epilachna borealis* on the radius (Text-fig. 1*C*). It may be no longer than No. 4 of *Epilachna*, or it may extend nearly all the distance to the fold of the wing (*Fo*). When two groups are present on each wing, one is similar to No. 2 of *Epilachna* and the other is similar to Nos. 3 and 4 united. The latter group may or may not extend all the way to the fold of the wing. In *Collops*, 20 pores were found on the ventral side of one wing besides the two groups on the dorsal surface. When three groups are found on each wing, they may be located like those of *Epilachna*, or two of them may lie on the radius and the third

one on the media. The largest one is similar to No. 4 of *Epilachna* and it may or may not extend all the way to the fold of the wing. When the third group lies on the media, as in *Orthosoma* (Plate II., Fig. 31), it occupies a position just beneath the larger group on the radius. Its pores are generally scattered considerably. When four groups are found on each wing, one of them lies on the subcosta, two on the radius and one on the media. It is common for the distal end of the largest group on the radius of any wing to become attenuated so that a row of pores may extend nearly, if not all, the way to the fold of the wing. The farther this row of pores extends along the radius the farther apart are the pores. It is also common for the largest group on the radius to consist of pores of two sizes. The diameters of the larger pores may be two or three times those of the smaller ones. The larger pores extend lengthwise through the center of the group. Eight species (Nos. 1, 3, 4, 5, 6, 33, 36, 38) have pores as just described. The pores in this group of seven other species (Nos. 10 to 14, 23, 30) are also of two sizes, but there is not such a great difference in the sizes of the smaller and larger pores, as in the pores of the preceding eight species. These pores are also comparatively larger. All the pores on the wings of nine species (Nos. 7, 9, 16, 17, 18, 20, 22, 31, 40) are of about the same size and they are comparatively large. All the pores on the wings of the remaining species are of about the same size, but they are comparatively small. *Coxelus*, the smallest beetle examined, with 130 pores on both wings has the least number, while *Orthosoma*, perhaps the largest beetle examined, with 982 pores on both wings has the greatest number.

The trochanters never fail to possess at least a few pores. The trochanter with the fewest pores has two, whereas the one with the most has 59. As a rule, the more pores on a trochanter, the smaller they are. The pores are generally located at the proximal end of this segment in about the same arrangement as represented in *Epilachna* (Text-fig. 2*A*–*F*), but occasionally they are considerably scattered, and a few may be found at the distal end of the segment.

A pore was found at the proximal end of one or more femurs belonging to each of 18 species (Nos. 1, 2, 4, 7, 8, 10, 11, 13, 16,

17, 18, 20, 21, 22, 24, 31, 48, 49), and from one to three pores were found at the proximal end of each femur of *Elater*.

While it is common to find one or more pores at the proximal end of a tibia, many of these segments are entirely devoid of olfactory pores. The greatest number of pores found on any tibia at this place is nine. In each of the tibio-tarsal articulations of the front and middle legs belonging to *Cotinis* from 7 to 11 pores were found. Pores were found in the tibial spines (Text-fig. 2*G* and Plate II., Fig. 27) of 15 species (Nos. 9 to 11, 20 to 25, 31, 32, 34, 35, 45, 48). The pores usually lie on the bases of the large spines. The largest number of pores found on a single tibial spine is 12. Of the 50 species examined, *Passalus* has the most pores on these spines.

Pores were found on the tarsi of 13 species (Nos. 1, 2, 4, 10 to 12, 16, 18 to 21, 25, 31). The greatest number found on a single tarsus was 37. *Osmus*, one of the three apterous species, has the most pores on its tarsi.

Eleven species (Nos. 6, 26, 27, 29, 31, 32, 33, 38, 42, 44, 47) were found with no pores on the legs except those on the trochanters and on the tibial spines. *Cybister* with 49 pores on all six legs has the least number on these appendages, while *Podabrus* with 341 pores on all six legs has the largest number.

No special examination was made to find any structure other than the olfactory pores, nevertheless, minute pores were seen in 15 species (Nos. 7, 8, 14, 15, 16, 20, 25, 27, 29, 36, 40, 44, 45, 46, 48). These pores were seen on various parts of the beetles, but particularly on the legs and elytra. They usually lie near the bases of the hairs, but sometimes they lie a considerable distance from the hairs. Since they are many times smaller than the olfactory pores, without exception they are probably the pores belonging to hypodermal glands, as will be shown for those of *Epilachna* on page 423. However, a careful comparative study of these pores is needed before anything definite can be said about them.

Coxelus, the smallest species examined, has a total number of 273 pores which is the smallest number of all the winged species, while *Orthosoma*, perhaps the largest species examined, has a total number of 1,268 pores, which is the largest number of all the

species examined. As a rule the smaller the species, the larger are the pores, comparatively speaking, and the fewer they are. Likewise, the larger the species, the greater is the number of its pores and the smaller they are. As a rule there are no generic and specific differences, except variations in number of pores, the amount of variation depending on the size of the individuals compared. Judging from the sizes of the four water beetles examined, the pores on their legs are fewer and smaller than those on the legs of any other beetle examined. Pores were found only on the trochanters of *Cybister*, while a few were also seen on the femurs and tibiæ of the other three water beetles. The number of pores on the legs of these beetles are as follows: *Cybister*—49, *Dineutes discolor*—65, *Dineutes vittatus*—98, and *Hydrophilus*—93. These numbers indicate that the better the legs are adapted for locomotion in water, the fewer pores they have.

The small total numbers of pores of *Osmus*, *Clinidium* and *Cysteodemus* are due to the absence of wings. In *Osmus* and *Clinidium* more pores are found on the legs than might be suspected. The tarsi of *Osmus* have more than the tarsi of any other beetle while the tarsi of *Clinidium* have more than suspected.

The following table (p. 419) includes the family, name and number, the olfactory pores on the legs, elytra, wings, and the total number of pores of each of the 50 species examined. In the preceding pages the beetles are usually referred to in this table by their respective numbers.

(*c*) *Individual and Sexual Variations.*

For this study five males and five females each of *Harpalus pennsylvanica* and *Leptinotarsa 10-lineata* were used. No individual and sexual variations were found, except slight variations in the number of pores. The total numbers of pores of the males of *Harpalus* vary from 550 to 580 with 570 as an average; those of the females of *Harpalus* from 575 to 699 with 628 as an average. The average number of pores for males and females of *Harpalus* is 599. The total numbers of pores of males of *Leptinotarsa* vary from 665 to 780 with 722 as an average; those of the females

TABLE I.

THE NUMBER OF OLFACTORY PORES ON THE LEGS, ELYTRA AND WINGS OF COLEOPTERA.

Family.	Name and Number of Species.	No. of Pores on Legs.	No. of Pores on Elytra.	No. of Pores on Wings.	Total No. of Pores.
Cicindelidæ.....	1. *Cicindela vulgaris*	180	29	924	1,133
	2. *Osmus* sp.	290	12		302
Carabidæ.....	3. *Calosoma scrutator*	140	62	869	1,071
	4. *Harpalus caliginosus*	180	69	600	849
	5. *Harpalus pennsylvanica*	107	39	453	599
Dytiscidæ.....	6. *Cybister fimbriolatus*	49	180	917	1,046
Gyrinidæ.....	7. *Dineutes discolor*	65	46	435	546
	8. *Dineutes vittatus*	98	42	532	672
Hydrophilidæ....	9. *Hydrophilus triangularis*	93	310	662	1,065
Silphidæ.....	10. *Necrophorus marginatus*	111	60	652	823
	11. *Silpha inæqualis*	118	30	664	812
Staphylinidæ ...	12. *Staphylinus macrulosus*	110	23	493	626
Scaphidiidæ.....	13. *Scaphidium quadguttatum*	111	96	185	392
Coccinellidæ.....	14. *Coccinella 9-notata*	93	132	310	535
	15. *Epilachna borealis*	95	149	288	532
Endomychidæ...	16. *Endomychus biguttatus*	144	31	178	353
Erotylidæ.....	17. *Megalodacne heros*	102	120	383	605
Colydiidæ.....	18. *Coxelus guttulatus*	93	50	130	273
Rhyssodidæ.....	19. *Clinidium sculptile*	131	40		171
Cucujidæ.....	20. *Cucujus clavipes*	107	104	325	536
Mycetophagidæ .	21. *Mycetophagus punctatus*	165	135	379	679
Dermestidæ.....	22. *Dermestes marmorata*	90	80	570	740
Histeridæ.....	23. *Hister depurator*	74	80	249	403
Trogositidæ.....	24. *Tenebroides castenea*	96	113	296	505
Elateridæ.....	25. *Elater apicatus*	111	130	365	606
Buprestidæ	26. *Melanophila longipes*	186	110	278	574
Lampyridæ.....	27. *Lucidota californica*	177	123	257	557
Telephoridæ.....	28. *Chaulcognathus pennsylvanica*	308	157	445	910
	29. *Podabrus comes*	341	157	280	778
Malachidæ.....	30. *Collops bipunctatus*	120	101	248	469
Lucanidæ.....	31. *Platycerus quercus*	158	160	536	854
	32. *Passalus cornutus*	203	184	782	1,169
Scarabæidæ.....	33. *Canthon lævis*	62	180	724	966
	34. *Cotinis nitida*	162	39	934	1,135
	35. *Euphoria sepulchralis*	90	36	613	739
	36. *Osmoderma scabra*	127	182	782	1,091
Cerambycidæ....	37. *Orthosoma brunneum*	79	207	982	1,268
	38. *Callimoxys fuscipennis*	87	175	510	772
	39. *Cyllene robiniæ*	110	40	629	779
Chrysomelidæ...	40. *Leptinotarsa 10-lineata*	115	130	476	721
Bruchidæ.....	41. *Bruchus pisi*	65	137	352	554
Tenebrionidæ....	42. *Tenebrio molitor*	66	145	462	673
	43. *Uloma impressa*	80	119	361	560
Meloidæ.....	44. *Cysteodemus armatus*	133	39		172
	45. *Epicauta marginata*	157	94	504	755
	46. *Epicauta pennsylvanica*	125	100	440	665
Rhipiphoridæ....	47. *Myodites scaber*	154	260	350	764
Rhynchophora:					
Rhynchitidæ. .	48. *Rhynchites bicolor*	84	148	480	712
Otiorhynchidæ	49. *Cephus latus*	71	111	382	564
Curculionidæ .	50. *Zygops seminivius*	65	116	392	573
Variation		49–341	12–310	130–982	273–1,268[1]

[1] The total number of pores of apterous species are not included.

of the same species from 661 to 785 with 720 as an average. It is thus seen that the females of *Harpalus* have a few more pores than the males, while the males and females of *Leptinotarsa* have the same number of pores.

Structure.

In the preceding pages it has been shown that most of the variations in regard to the disposition of the olfactory pores are slight. In the following pages it will be shown whether or not this is true for the structure of these pores.

(*a*) *External Structure.*

When examined under a low-power lens, the olfactory pores may be easily mistaken for hair sockets from which the hairs have been removed. When more carefully observed under a high-power lens, a striking difference in external form is usually seen, but sometimes it is difficult to distinguish the pores from hair sockets. The pores appear as small bright spots when a strong transmitted light is used. Each bright spot has a dark boundary or pore wall (Plate I., Fig. 1, *PorW*).[1] Near the center of this boundary is a transparent spot, the pore aperture, which may be round, oblong, slit-shaped, or club-shaped. On the legs the pore apertures may be round (Fig. 2, *PorAp*), oblong (Fig. 3, *PorAp*), slit-shaped or club-shaped (Fig. 1, *PorAp*). On the elytra and wings they may be round or oblong (Figs. 4 to 8). The hair sockets (Figs. 1 and 2, *PorWHr*) are generally smaller than the olfactory pores and the pores of the hypodermal glands (Figs. 1 and 2, *PorWGl*) are easily distinguished from the hair sockets and olfactory pores by their small size.

(*b*) *Internal Structure.*

All the olfactory pores studied are more or less flask-shaped structures. They are of three general types. In the most common type, as found in *Uloma*, the mouth of the pore (Figs. 9–12, *Mo*) is flaring and the sense cell (Fig. 12, *CS*) lies in the lumen of the appendage outside the pore cavity. The chitinous

[1] All figures, except Text-figs. 1, 2, and 3 are numbered consecutively on Plates I. and II.

cone (Fig. 9, *Con*) never occupies more than one fourth of the pore cavity and usually much less (Fig. 12, *Con*). The cone always stains less deeply than the surrounding chitin, and it is common to see a hypodermal secretion (Figs. 9 and 10, *HypS*) inside the pore cavity. The sense fiber (Fig. 9, *SF*) pierces the cone, and the chitin between the pore aperture and the cone, and it ends in the bottom of the pore aperture or pit (Figs. 9–12, *P*) with its peripheral end exposed to the air in the pit.

The second type of pores is found in the legs of *Orthosoma* (Figs. 13–15), although the pores in the elytra (Fig. 21) and wings (Fig. 31) of the same beetle belong to the first or most common type. The chitinous integument of the legs of *Orthosoma* is thicker than that of the legs of any other beetle examined. Instead of the sense cells (Fig. 13, *SC*) lying in the lumen of the legs outside the pore cavities, in this type they lie inside the pore cavities. When the chitin forming the wall of the pore is not thick enough to protect the entire sense cell, the wall of the pore projects flange-like (Fig. 14, *Fl*) into the lumen of the leg. In Fig. 14 only about one third of the sense cell (*SC*) is shown. Studies of the olfactory pores in various hymenopterous insects made by the writer have shown that the sense cells begin to differentiate at the time when the chitin is beginning to be formed. From this fact, it is quite probable that the sense cells found in the second type of pores have not migrated into the pore cavities, but they now remain in approximately the same position as when the chitin was being formed.

The third type of pores is found in the legs of the lady beetle, *Epilachna borealis*. Instead of the chitin over the external end of the pore being depressed to form a pit, it is elevated dome-like above the surface of the leg. In the center of the dome lies the pore aperture (Fig. 16, *PorAp*). All the pores in the trochanters and most of those in the tibiæ (Fig. 17, *PorAp*) are of this type. Sometimes in the tibia is found a pore whose aperture is on a level with the surface of the tibia. The apertures of all the pores in the elytra (Fig. 18, *PorAp*) and wings (Fig. 19) of this beetle are on a level with the surfaces of the appendages.

As already stated, the olfactory pores of beetles are more or less flasklike as a rule, but there are many variations among

them. They may be inverted flask-shaped as found in the legs of *Epilachna* (Figs. 16 and 17) and in the wings of *Passalus* (Fig. 20). Some have the shape of a flask without the neck (Figs. 9, 10 and 12). Some are long and slender like fingers or test tubes (Figs. 11, 18, 19 and 21).

Their sizes also vary much. The length of a pore always depends on the thickness of the chitin. The diameters of the pores of a small beetle (Fig. 25) may be as large, or even larger (Figs. 9 and 10) than the diameters of the pores of a large beetle (Figs. 13–15).

A chitinous cone is always present, although it may sometimes be almost indiscernible. It invariably has the same shade of coloration (Fig. 17, *Con*) as the remaining chitin (Fig. 17, Ch_2) which is formed after the insect has emerged into the imago stage. This is the first time that the writer has been able to determine definitely the formation of the cones. In all the hymenopterous insects studied by the writer, the chitinous integument is practically developed when the insects emerge, but in most beetles only about one third of the chitin is formed when the insects emerge. Since this is true the hypodermal cells are still large and they are rapidly secreting a substance which forms new chitin. Their external ends stand in contact with the chitin, and when no chitin is present they send processes into all holes or cavities in the chitin. Thus the hypodermal cell (Fig. 23, *HypC*) at the mouth of each olfactory pore sends a process into the pore. Since the sense fiber has entered the pore aperture before the cone is formed, the latter is formed at the external end of the pore around the sense fiber. When the chitinous integument (Fig. 17) is fully developed no hypodermal processes run into the pores and the hypodermal cells are very small.

The sense cells are always spindle-shaped (Figs. 12, 13, 16–19 and 23, *SC*). Only occasionally is an entire sense cell seen in a cross section, because the entire cell seldom lies in the same plane as that of the section. More entire sense cells may be seen in longitudinal sections, but even in these the cells are usually cut in two. Entire sense cells were best seen in the oblique sections through the peduncles of the elytra of *Passalus* and *Epilachna*. The nucleus (Figs. 13 and 23, *SCNuc*) of the sense cell is always

conspicuous. It may be darker (Fig. 13, *SCNuc*) or lighter (Fig. 23, *SCNuc*) in color than the cytoplasm in the cell. The nucleoli (Fig. 23, *SCNuc*) are also conspicuous.

Smaller sense cells may be seen in the sections through the proximal ends of the trochanters and through the proximal ends of the tibiæ. These (Fig. 17, SC_1) belong to tactile hairs (Fig. 17, *THr*).

In the sections through the legs and elytra of *Epilachna*, gland cells (Fig. 17, *GlC*) are plainly seen in the hypodermis (*Hyp*). These are equally as large as the olfactory sense cells, but they are quite different in structure. The diameters of the pores of the glands (*PorGl*) are slightly smaller than those of the hairs (*PorHr*), and they are much smaller than those of the olfactory pores (*Por*). The morphology and physiology of these gland cells will be given in another paper.

The shapes of the external ends or tops of the pits depend on the shapes of the pore apertures when seen in superficial views. That is, they are round, oblong, slitlike or clublike. The internal ends or bottoms of the pits are always round. The pore aperture, proper, is the round opening leading from the bottom of the pit to the external end of the pore. This aperture is closed by the peripheral end of the sense fiber. The shapes of the pits in cross sections, therefore, depend on the directions in which the microtome knife passes through the pits. The most common shape of a pit in cross section is that of an urn (Fig. 9, *P*). Pits including the pore apertures may be likened to round funnels, or to funnels slightly flattened, or to funnels considerably flattened, or to funnels so flattened that their tops would be club-shaped. In spiders the pits are slits which pass entirely through the cuticula. The sense fibers enter the pore apertures at the bottoms of the slits. The pits or slits in spiders, therefore, may be likened to funnels considerably flattened. When just emerged into the imago stage the pits (Fig. 9, *P*) in the legs generally extend about one-third the distance through the chitin, but when the chitin is fully developed, the pits extend perhaps from one fifth to one eighth the distance through the chitin. In all the figures showing two shades in the chitin, the darker one (Fig. 17, Ch_1) represents the chitin formed at the time when the

insect emerges from the last pupal stage, and the lighter one (Ch_2) represents the chitin formed after emerging into the imago stage.

As already stated, instead of the olfactory pores of the lady beetle, *Epilachna*, having pits, the chitin over each pore in the legs is elevated domelike above the surface of the leg. The olfactory pores (Figs. 24 and 25) in the legs of the two blister beetles, *Epicauta marginata* and *Epicauta pennsylvanica*, have only indications of pits. Their pore apertures are therefore on a level with the surface of the legs. The olfactory pores in the legs of the potato beetle, *Leptinotarsa 10-lineata*, have shallow pits (Fig. 26, *P*). All four just enumerated species have hypodermal gland pores distributed over the entire body except the wings. These pores are perhaps most abundant on the elytra, but they were never seen on the peduncles of these appendages, and it is quite probable that the secretion from their glands never covers the olfactory pores found on the wings and on the peduncles of the elytra. Judging from the gland pores, the hypodermal glands in the legs of *Epilachna* are more highly developed than are those of the other three species. The gland pores (Figs. 1, 2 and 28, *PorWGl*) on the legs of *Epilachna* lie on all sides and even among the olfactory pores, but in the legs of the other three species the gland pores never lie near the olfactory pores. When examined under a low-power lens the legs and elytra of *Epilachna* appear wet, and many small yellow flakes may be seen on them. The wet appearance is certainly due to the secretion from the hypodermal glands and the flakes are the remains of the secretion after it becomes dry. Thus in *Epilachna* there seems to be a direct correlation between the olfactory pores and the gland pores. Since the pore apertures in the legs lie above the surface of these appendages, the secretion from the hypodermal glands runs away from the pore apertures instead of into them. Such a device enables both sets of organs to function normally without the one hindering the other.

In the legs the sense cells always lie in a blood sinus (Figs. 16 and 17, *BlSin*) some distance from the muscles (Fig. 28, *M*). The nerves (*N*) are easily seen and branches (*NB*) are given off which run to the sense cells (*SC*). The neurilemma (Fig. 17,

Neu) of the nerve is usually distinct. In the cross section of a nerve, the nervous substance appears more or less netlike and nuclei, probably neuroglia nuclei (Fig. 17, *NeurNuc*), stand out conspicuously in the network. The trachea (Figs. 16, 17 and 28, *Tr*) and nerves (*N* and *NB*) are firmly suspended by the connective tissue whose nuclei (*ConTNuc*) are seen only occasionally. The lumen of the leg at the proximal end of the tibia of *Epilachna* seems to be divided into two chambers by a membrane (Fig. 17, Hyp_1) which resembles hypodermis. This structure has never been seen before by the writer and nothing can be said about its function.

The hypodermis (Fig. 18, *Hyp*) beneath the olfactory pores in the peduncles of the elytra is much thicker than elsewhere. It usually contains all the sense cells (*SC*), but in the elytra of *Passalus* the hypodermis is thinner and since the sense cells are so large and so numerous there is not enough room for all of them in the hypodermis. For this reason only a few of them lie among the hypodermal cells and the remainder of them lie in the lumen of the peduncle between the hypodermis and nerve. As usual they are surrounded by blood. In only one instance was the writer able to trace a sense cell all the way from the pore aperture to the nerve. Fig. 23 represents this sense cell connecting with the pore aperture (*PorAp*) and with the nerve (*N*). The trachea (*Tr*) lies by the side of the nerve. A large nerve (Fig. 29, *N*) and a large trachea (*Tr*) run through the radial plate (*RP*) of the peduncles beneath the olfactory pores. From the nerve many branches are given off which connect with the sense cells.

The hypodermis (Fig. 22, *Hyp*) beneath the olfactory pores in the wings is usually much thicker than elsewhere, but it does not contain the sense cells (*SC*). These cells lie in a blood sinus (Fig. 22, *BlSin*) between the hypodermis (*Hyp*) and the trachea (*Tr*), nerve (*N*) and nerve branches (*NB*). In the wings it is usually difficult to trace a sense fiber all the way to the pore aperture, but in oblique superficial sections this is easily done (Fig. 30). A large nerve and a large trachea run into each wing. These divide so that a smaller nerve and a smaller trachea run through each main vein. The largest trachea (Fig. 31, *Tr*) runs

through the subcosta (*Sc*) while the largest nerves (*N*) pass through the veins bearing the olfactory pores. The nerve and trachea run directly beneath the sense cells (*SC*) and from the nerve pass off many branches which connect with the sense cells. In the costa (*C*) and subcosta (*Sc*) where there are no sense cells, only a few nerve fibers can be seen.

In the preceding pages it has been shown that there are many variations in the structure of the olfactory pores of beetles, and that these organs are very similar to those of hymenopterous insects. On the basis of the location of the pore apertures in the integument, the olfactory organs in beetles are intermediate between those of spiders and those of Hymenoptera.

EXPERIMENTS TO DETERMINE THE LOCATION OF THE OLFACTORY ORGANS.

Since it is now generally believed that the olfactory organs of beetles are borne by their antennæ, these appendages of many individuals were pulled off. From one to seven days later, the mutilated insects were tested with odors. In the preceding pages it has been shown that the olfactory pores of Coleoptera are located on the peduncles of the elytra, on the wings and on the legs. In order to ascertain if these structures receive odor stimuli, the elytra, wings and legs were mutilated. One or more days later these mutilated beetles were tested with odors. In all the experiments with unmutilated and mutilated beetles, 434 individuals have been tested. These belonged to 11 species representing eight families.

In order that the behavior of the mutilated beetles would be correctly interpreted, the behavior of unmutilated beetles under experimental conditions was first studied. Since it was not desired to ascertain the relative sensitiveness of males and females, both sexes were used indiscriminately. To determine the relative sensitiveness of unmutilated and mutilated individuals under conditions which permitted of their close observation, triangular experimental cases were employed. These were made of three narrow wooden strips, two of which were five and the third four inches long, each strip being half an inch thick. Wire screen served as a bottom and glass as a top for the case.

The apices and bases of these cases rested on two supports above a rigid table near a window. No screen was used to prevent the beetles from seeing the observer because they never showed any responses to the movements made by the observer.

The following sources of odors were used for determining the reactions of the beetles in the experimental cases; chemically pure essential oils of peppermint, thyme, and wintergreen; parts of plants—leaves and stems of pennyroyal (*Hedoma pulegioides?*), and of spearmint (*Mentha spicata*); decayed matter —parts of decayed beetles (*Harpalus pennsylvanica*). All these substances were kept in stoppered vials of the same shape and size. The leaves and stems of the pennyroyal were dried, but they still gave off a strong odor when the vial was uncorked. The leaves and stems of the spearmint were fresh and they did not emit as strong an odor as did the other substances used. Beetles were killed and were torn to pieces. The pieces were put into a vial and after two or three days they emitted a foul and sickly odor.

A beetle was carefully placed into one of the experimental cases. When first put into the case the insect usually wandered about for several minutes, but finally it became quiet. The insect was tested with the above odors only when it had become perfectly quiet, without the antennæ being moved in the least. The stopper of a vial was quickly removed and the vial was gently and slowly placed under the experimental case directly beneath and within one half inch of the individual being tested. When all of these precautions are taken, a normal beetle generally responds to anyone of these odors within 60 seconds, but when all the reaction times are counted, it is seen that several of them failed to respond within 60 seconds. If a beetle when tested fails to react to an odor within 60 seconds, the response may be regarded as negative, and when it reacts to an odor within 60 seconds, the response may be called positive. As a control, an empty and odorless vial was now and then placed under the insects in the same manner. If by chance a beetle moved while the control test was being made, its behavior was different from that observed when odors were used. Only the first responses have been recorded and in all cases where there was the least

doubt as to whether the insect moved for any reason other than the olfactory stimulus, such movements were never recorded. The reaction time was counted in seconds. With an ordinary watch the minimum time which can be definitely recorded is two seconds, although many of the individuals responded to some of the odors much more promptly. Owing to this source of error, the average recorded time is probably double what it should be in the cases where all the responses for the same insect were prompt. An intermission of 10 minutes elapsed between any two tests in the same experimental case. Each individual was tested only once with the same odor.

In recording the responses the term "vibrated" is used to describe the rapid movement of the antennæ or legs up and down or from side to side. When this movement is slow, these appendages are described simply as having "moved." When the antennæ, legs or mouth parts are moved so that they are quickly bent at their articulations, they may be described as being "worked." When at rest a beetle usually lies flat on its thorax and abdomen, so the word "arose" means that the insect gets up and stands on its feet. In the averages of reaction times the probable error is presumably high. It has not been calculated since slight differences in reaction times are not considered as significant in the discussion of results. All anthropomorphic terms are put in quotation marks.

CARABIDÆ.

The Olfactory Sense of *Harpalus pennsylvanica*.

Many ground beetles (*Harpalus pennsylvanica*) were caught under flat stones in a corn field near the laboratory. As soon as brought to the laboratory, 25 of them were placed singly into the experimental cases. As they were being placed into the cases, some of them discharged a substance, presumably from the anal glands, which gave off an odor similar to that from formic acid. Confined in these cases, they sought the dark corners of the cases and did not wander about much inside the cases unless irritated. When half hidden in the dark corners, they rarely responded to odors, so it was necessary to keep them out of the corners while they were being tseted. The longer they remained

in the light and the more they were handled, the more satisfactory they were to experiment with. Owing to this kind of behavior, this species and several others used responded more slowly to odors a short time after being caught than they did a few days after being kept in confinement. This fact will explain why some unmutilated beetles just caught respond to odors more slowly than they do two or three days later after having had their antennæ pulled off. The following are the responses of this ground beetle to the odors from the six different substances and the average reaction times in seconds.

Oil of peppermint:

5 moved away quickly.	1 worked legs.
5 vibrated antennæ.	1 kicked quickly.
4 arose quickly.	1 vibrated antennæ and legs.
4 moved slightly.	1 vibrated legs.
2 moved antennæ and legs.	1 jumped slightly.

Reaction time 2 to 10 seconds, average 3.6 seconds.

Oil of thyme:

6 moved away quickly.	1 arose slowly.
5 moved quickly.	1 vibrated antennæ.
5 moved slightly.	1 moved backward slowly.
2 worked antennæ.	1 worked legs.
2 moved antennæ and legs.	1 did not respond.

Reaction time 2 to 60 seconds, average 8.5 seconds.

Oil of wintergreen:

5 moved away quickly.	1 stroked antennæ.
5 moved slightly.	1 vibrated antennæ and legs.
4 moved away slowly.	1 worked antennæ.
3 moved quickly.	1 worked legs.
2 vibrated legs.	1 did not respond.
1 arose slowly.	

Reaction time 2 to 60 seconds, average 16.4 seconds.

Leaves and stems of pennyroyal:

10 moved away quickly.	1 moved away slowly.
6 moved slightly.	1 vibrated legs.
3 vibrated antennæ.	1 did not respond.
3 worked antennæ.	

Reaction time 3 to 60 seconds, average 21.1 seconds.

Leaves and stems of spearmint:

5 moved away slowly.	2 did not respond.
5 moved slightly.	1 worked mouth parts.
3 moved antennæ and legs.	1 vibrated legs.
2 moved away quickly.	1 vibrated antennæ and legs.

2 worked antennæ.
2 jumped slightly.
1 moved antennæ.

Reaction time 3 to 60 seconds, average 21.8 seconds.

Parts of decayed beetles:

7 moved slightly.
5 did not respond.
4 moved away quickly.
3 moved away slowly.
2 jumped slightly.
1 moved legs.
1 worked antennæ.
1 vibrated legs.
1 vibrated antennæ and worked mouth parts.

Reaction time 5 to 60 seconds, average 28.1 seconds.

The general average reaction time of the 25 beetles tested to the six odors is 16.5 seconds. As a possible reason why one fifth of the individuals tested failed to respond to the odor from the decayed beetles is that these insects probably do not respond to decayed matter unless they are hungry. The 25 beetles tested were put into a wooden box four inches wide, seven inches long and two inches deep. One half inch of moist earth was also put into the box. The beetles soon buried in the earth and from that time on they appeared quite "at home." The box was put into a table drawer where it was more or less dark. About twice each week water was poured upon the earth and the beetles were fed earthworms and various insect larvæ. They drank some of the water and always greedily ate the food given to them. Up to the time of this writing (Jan. 15), 24 of these beetles have died. These lived from 18 to 180 days with 61 days as an average. All the beetles confined in the laboratory have not been fed since Oct. 15, but they have been given water once or twice a week. A few of the dead beetles when removed from the box had been partially eaten, but these insects were never seen fighting one another. While collecting this species in the corn field, a dead one was now and then found.

(*a*) *Effects with Antennæ Pulled Off.*

The antennæ of 25 *Harpalus pennsylvanica* were pulled off at their bases. These insects were then put into a wooden box similar to the one containing the unmutilated individuals just described. This box, also containing moist earth, was placed into the table drawer. The beetles appeared normal in all respects for they drank and ate as greedily as the unmutilated

ones and buried in the earth as usual. Seven days later they were placed singly into the experimental cases and were tested with the six odors as usual. They wandered about in the cases slightly more than did the unmutilated ones, but when tested they gave similar responses and reacted just as promptly.

Their reaction times are as follows: Oil of peppermint, 2 to 15 seconds, average 3.8 seconds; oil of thyme, 2 to 25 seconds, average 4.7 seconds; oil of wintergreen, 2 to 25 seconds, average 6.9 seconds; leaves and stems of pennyroyal, 3 to 50 seconds, average 14.4 seconds; leaves and stems of spearmint, 3 to 60 seconds, average 34.9 seconds. Ten failed to respond to this odor. Parts of decayed beetles, 3 to 60 seconds, average 32 seconds. Eight failed to respond to this odor. The general average reaction time of the 25 beetles tested to the six odors is 16.1 seconds. Up to the time of this writing (Jan. 15), 23 of these beetles have died. They lived from 19 to 171 days with 58 days as an average.

(*b*) *Effects with Elytra and Wings Pulled Off.*

The elytra and wings of 25 *Harpalus pennsylvanica*, just collected from the cornfield, were pulled off at their articulations. These mutilated insects were then put into a third box, similar to the two already described. The box was kept in the table drawer with the others. On the following day after mutilating the beetles, they were placed singly into the experimental cases and were tested with the six odors as usual. They seemed normal in all respects except they were extremely restless. Their responses to odors were similar to those of unmutilated ones, except they were slower.

Their reaction times are as follows: Oil of peppermint, 3 to 45 seconds, average 10.7 seconds; oil of thyme, 5 to 50 seconds, average 10.2 seconds; oil of wintergreen, 5 to 60 seconds, average 18 seconds. Two failed to respond to this odor. Leaves and stems of pennyroyal, 5 to 60 seconds, average 29.2 seconds. Seven failed to respond to this odor. Leaves and stems of spearmint, 5 to 60 seconds, average 24.7 seconds. Four failed to respond to this odor. Parts of decayed beetles, 5 to 30 seconds, average 13.4 seconds. The general average reaction time of the

25 beetles tested to all six odors is 17.7 seconds. These mutilated insects lived from 2 to 21 days with 9 days as an average. All the time they were confined in the small box, they drank, ate, and buried in the earth normally, but many times one was seen biting the soft dorsal portion of the abdomen of another. With the elytra and wings removed, the abdomens were unprotected and many of them shrank considerably in size before the beetles died. Some of these beetles were certainly killed on account of the dorsal sides of their abdomens being bitten, because nearly every one found dead had been entirely eaten except the chitinous parts. In the other two boxes as already mentioned, only occasionally was a dead beetle found that had been eaten.

(*c*) *Effects with Elytra and Wings Pulled Off and Pores on Legs Covered with Vaseline.*

The elytra and wings of 18 *Harpalus pennsylvanica* were pulled off at their articulations. Four days later the trochanters, femurs and proximal ends of the tibiæ of these mutilated beetles were covered with a vaseline-beeswax mixture, consisting of three fourths yellow commercial vaseline and one fourth beeswax. An hour after the legs had been vaselined, the beetles were placed singly into the experimental cases and were tested with the six odors as usual. Most of them were comparatively quiet, but a few were extremely restless. Their responses to odors were not pronounced and were slow, otherwise they were similar to those of unmutilated beetles.

Their reaction times are as follows: Oil of peppermint, 3 to 60 seconds, average 19.5 seconds. Three failed to respond to this odor. Oil of thyme, 3 to 60 seconds, average 12.5 seconds. Two failed to respond to this odor. Oil of wintergreen, 3 to 60 seconds, average 18.7 seconds. Four failed to respond to this odor. Leaves and stems of pennyroyal, 5 to 60 seconds, average 38.6 seconds. Nine failed to respond to this odor. Leaves and stems of spearmint, 3 to 60 seconds, average 32.9 seconds. Seven failed to respond to this odor. Parts of decayed beetles, 4 to 60 seconds, average 22.1 seconds. Two failed to respond to this odor. The general average reaction time of the 18 beetles tested to all six odors is 24.1 seconds. Confined in a box similar

to the other three already mentioned, these mutilated beetles drank, ate and buried in the earth normally, but they were less active than unmutilated ones. It was common to see them biting the dorsal sides of the abdomens. Before they died several of their abdomens had shrunk considerably in size. When found dead several of them had been entirely eaten except the chitinous parts. Counting from the time the elytra and wings were pulled off, they lived from 5 to 21 days with 10 days as an average.

The Olfactory Sense of *Harpalus caliginosus*.

Eight ground beetles (*Harpalus caliginosus*) were caught under flat stones. They were tested with the odors from only the three essential oils. In behavior, they were comparatively quiet. When tested, many of them moved away quickly; a few vibrated the antennæ, and a few moved their legs.

Their reaction times are as follows: Oil of peppermint, 2 to 10 seconds, average 4.4 seconds; oil of thyme, 2 to 8 seconds, average 4.1 seconds; oil of wintergreen, 2 to 8 seconds, average 4.1 seconds. The general average reaction time to all three odors is 4.2 seconds. The antennæ of these beetles were pulled off and the insects were then kept in a small box containing earth in the table drawer.

(*a*) *Effects with Antennæ Pulled Off.*

Eight days after the antennæ of the eight preceding *Harpalus caliginosus* had been pulled off, the remaining six live ones were again tested with the same odors in the usual way. Their responses were similar to those given before they were mutilated, but were not so pronounced. When tested with the oil of thyme, one beetle rubbed a hind leg on an elytron for a half minute.

Their reaction times are as follows: Oil of peppermint, 3 to 25 seconds, average 12.5 seconds; oil of thyme, 4 to 60 seconds, average 14.3 seconds. One failed to respond to this odor. Oil of wintergreen, 10 to 35 seconds, average 22.5 seconds. The general average reaction time to all three odors is 16.4 seconds. These mutilated beetles were quite inactive and sometimes scarcely moved when touched with a pencil. They did not eat as greedily as before being mutilated. They lived from 2 to 65 days with 18 days as an average.

COCCINELLIDÆ.

The Olfactory Sense of *Epilachna borealis*.

Many lady beetles (*Epilachna borealis*) were caught on pumpkin vines in the corn field. When brought to the laboratory, they were put into a large glass jar near a window. The jar was 11 inches tall and 9 inches in diameter. It was covered with cheesecloth. Since this lady beetle feeds upon the leaves of pumpkin and of allied plants, several pumpkin leaves were put into a wide-mouthed bottle containing water. The bottle with contents was then put into the jar. The beetles soon found the leaves and from that time on, they appeared "at home" as much as they do in corn fields on pumpkin leaves. They were regularly provided with a fresh supply of food. Occasionally they were seen copulating.

On the following day after being caught, 18 of them were removed from the jar and were put singly into the experimental cases. When mechanically irritated they draw in the antennæ and legs, usually eject a small drop of yellowish liquid from each femoro-tibial articulation, and feign death. They may lie apparently lifeless for several moments and when tested with odors they may or may not respond. Owing to this peculiar behavior, they were unsatisfactory to experiment with and their average reaction times are slower than might be expected. They were extremely quiet and when tested they generally moved away slowly. They often vibrated the antennæ and mouth parts, and sometimes the legs.

Their reaction times to the odors from the three essential oils are as follows: Oil of peppermint, 2 to 55 seconds, average 12.4 seconds; oil of thyme, 2 to 20 seconds, average 6.8 seconds; oil of wintergreen, 3 to 60 seconds, average 22.2 seconds. Three failed to respond to this odor. The general average reaction time to all three odors is 13.8 seconds. Sixteen of these insects were mutilated for other experiments. The seventeenth lived only 3 days and the eighteenth is still living at this writing (Jan. 15).

(*a*) *Effects with Antennæ Pulled Off.*

The antennæ of 25 *Epilachna borealis*, just caught, were pulled off at their bases. A small drop of yellowish blood exuded from

each wound. On the following day the beetles were tested with odors. As a rule they were so inactive that they appeared lifeless. If touched while moving they feigned death and remained inactive for several moments. When tested with odors most of them worked the mouth parts; some moved away slowly; a few vibrated one or more legs, and some failed to respond.

Their reaction times to the odors from the three essential oils are as follows: Oil of peppermint, 2 to 60 seconds, average 18.6 seconds. Three failed to respond to this odor. Oil of thyme, 2 to 60 seconds, average 38.7 seconds. Fourteen failed to respond to this odor. Oil of wintergreen, 3 to 60 seconds, average 35.1 seconds. The general average reaction time to all three odors is 30.8 seconds. Up to the time of this writing (Jan. 15), 15 of these mutilated beetles have died. They lived from 1 to 96 days with 22 days as an average.

(*b*) *Effects with Elytra and Wings Pulled Off.*

The elytra and wings of 10 *Epilachna borealis* were pulled off at their articulations. A small drop of yellowish blood exuded from each wound. A liquid of the same color is also present throughout the elytra and in the veins of the wings. On the second day after being mutilated, the four remaining live beetles were tested as usual. They were very quiet, but appeared normal in all respects except they responded to odors more slowly than unmutilated ones.

Their reaction times to the odors from the three essential oils are as follows: Oil of peppermint, 10 to 60 seconds, average 25 seconds. One failed to respond to this odor. Oil of thyme, 5 to 60 seconds, average 33.5 seconds. Two failed to respond to this odor. Oil of wintergreen, 7 to 60 seconds, average 35.5 seconds. Two failed to respond to this odor. The general average reaction time to all three odors is 31.3 seconds. Up to the time of this writing (Jan. 15), 1 of these beetles has died. Counting the 7 mutilated beetles that died, they lived from 2 to 3 days with 2 days as an average.

TELEPHORIDÆ.

THE OLFACTORY SENSE OF *Chaulcognathus pennsylvanica*.

Many fireflies (*Chaulcognathus pennsylvanica*) were caught on goldenrod (*Solidago*). They were put into a cage 20 inches long, 16 inches tall and 12 inches wide. The sides and top of the cage were cheesecloth while the ends and bottom were wood. The cage was kept in the light near a window and a fresh supply of goldenrod was constantly kept in the cage. On the goldenrod in the cage, these insects appeared quite "at home." Twenty-five of them were tested with the odors from the three essential oils. When tested most of them moved away quickly; a few vibrated antennæ; a few vibrated legs, and a few arose slowly. They were extremely restless at all times. In the cage they copulated as freely as they do out-of-doors.

Their reaction times are as follows: Oil of peppermint, 2 to 12 seconds, average 2.6 seconds; oil of thyme, 2 to 10 seconds, average 3 seconds; oil of wintergreen, 2 to 10 seconds, average 3 seconds. The general average reaction time to all three odors is 2.8 seconds. They lived from 3 to 7 days with 3.2 days as an average.

(*a*) *Effects with Antennæ Pulled Off.*

The antennæ of 27 *Chaulcognathus pennsylvanica* were pulled off at their bases. A day later only three were alive. When tested these three responded as promptly as unmutilated ones. The general average reaction time to the odors from the three essential oils is 2.8 seconds. Counting all 27 beetles, they lived from 1 to 5 days with 1.3 days as an average.

LUCANIDÆ.

THE OLFACTORY SENSE OF *Passalus cornutus*.

Four stag beetles (*Passalus cornutus*) were removed from rotten stumps. While being tested with odors they were comparatively quiet and responded promptly. Their most common response was to draw in the antennæ and to move away slowly. The general average reaction time to all six odors is 3.2 seconds. The antennæ were pulled off at their bases. A small drop of

blood exuded from each wound. The beetles were kept in a small box filled with moist rotten wood.

(*a*) *Effects with Antennæ Pulled Off.*

Two days after pulling off the antennæ, the four preceding mutilated beetles were again tested with the same odors. They were more quiet than before being mutilated. Their responses were just as prompt but were less pronounced than before they were mutilated. Their most common response was to work the mouth parts and to move away slowly. The general average reaction time to all six odors is 3.3 seconds. They lived from 4 to 20 days with 12.5 days as an average.

SCARABÆIDÆ.

THE OLFACTORY SENSE OF *Cotinis nitida.*

One lamellicorn beetle (*Cotinis nitida*) was tested with the six odors. The most common response was to stretch out its head, and to move its antennæ and front legs. Once it drew in the antennæ and moved the front legs. The average reaction time is 8 seconds. The antennæ were pulled off at their bases. A small drop of blood exuded from each wound.

(*a*) *Effects with Antennæ Pulled Off.*

A day after pulling off the antennæ, the preceding *Cotinis nitida* was again tested with the same odors. It responded as promptly as before being mutilated. The most common response was to work the mouth parts and to move away slowly. The average reaction time is 8.3 seconds. It lived 12 days after being mutilated.

THE OLFACTORY SENSE OF *Euphoria sepulchralis.*

Five lamellicorn beetles (*Euphoria sepulchralis*) were caught on goldenrod (*Solidago*). While being tested with the odors from the three essential oils, they were extremely restless. They generally moved away slowly and drew in the antennæ when tested with an odor. The general average reaction time is 3.6 seconds. After the antennæ had been pulled off at their bases, the beetles were put into the cage described on page 436.

(*a*) *Effects with Antennæ Pulled Off.*

A day later the five preceding mutilated insects were again tested with the same odors. They were quiet and their responses were similar to those before being mutilated, except, of course, there were no antennal movements. The general average reaction time is 4.3 seconds. These beetles lived from 9 to 42 days with 20 days as an average after being mutilated.

CERAMBYCIDÆ.

THE OLFACTORY SENSE OF *Cyllene robiniæ*.

Eighteen wood-boring beetles (*Cyllene robiniæ*) were caught on goldenrod (*Solidago*). While being tested with the odors from the three essential oils, they were extremely restless. When tested, most of them moved away quickly; a few arose quickly, and a few vibrated the antennæ. The general average reaction time is 5.4 seconds. These beetles were confined in the cage described on page 436. They were regularly given a fresh supply of goldenrod. They seemed "at home" and copulated as freely in the cage as they do out-of-doors. They lived from 1 to 17 days with 10.4 days as an average.

(*a*) *Effects with Antennæ Pulled Off.*

Eighteen more *Cyllene robiniæ* were collected from goldenrod. Their antennæ were pulled off at the bases. A small drop of blood exuded from each wound. These beetles were placed into the cage with the unmutilated ones. Two days later the 15 remaining live ones were tested with the odors from the essential oils. They were very quiet and their responses were similar to those of unmutilated individuals, except as a rule they were more prompt. The general average reaction time is 3 seconds. In the cage it was common to see the unmutilated and antennæless cerambycids copulating. The former were very active and flew out of the cage whenever the door was opened, but the latter seldom flew and they were not so active. The mutilated ones lived from 1 to 11 days with 5 days as an average.

(*b*) *Effects with Elytra and Wings Pulled Off.*

Eighteen more *Cyllene robiniæ* were collected. Their elytra and wings were pulled off at the articulations. A small drop

of blood always exuded from each wound caused by the elytron being pulled off, but only occasionally was blood seen where a wing had been pulled off. A day later when tested with the odors from the three essential oils, these beetles were comparatively quiet and they appeared normal in all respects except in their slowness in responding to odors.

Their reaction times are as follows: Oil of peppermint, 2 to 30 seconds, average 7.1 seconds; oil of thyme, 3 to 20 seconds, average 8.9 seconds; oil of wintergreen 3 to 55 seconds, average 13.4 seconds. The general average reaction time to all three odors is 9.8 seconds. In the cage with the other beetles, these mutilated ones were as active as the unmutilated cerambycids and they were often seen copulating with each other, and with the unmutilated and antennæless ones. They lived from 1 to 11 days with 4.2 days as an average.

CHRYSOMELIDÆ.

The Olfactory Sense of *Leptinotarsa 10-lineata*.

Forty-five Colorado potato beetles (*Leptinotarsa 10-lineata*) were collected in a potato patch near the laboratory. While 25 of them were being tested with the six odors, they were comparatively quiet as a rule, but five were so restless that they were discarded and others were used. Their responses were similar to those of *Harpalus pennsylvanica*, described on page 429.

Their reaction times are as follows: Oil of peppermint, 2 to 7 seconds, average 3.3 seconds; oil of thyme, 2 to 5 seconds, average 3.1 seconds; oil of wintergreen, 2 to 12 seconds, average 5 seconds; leaves and stems of pennyroyal, 4 to 60 seconds, average 26.7 seconds. Six failed to respond to this odor. Leaves and stems of spearmint, 2 to 60 seconds, average 25.6 seconds. Seven failed to respond to this odor. Parts of decayed beetles, 5 to 60 seconds, average 27.9 seconds. Seven failed to respond to this odor. The general average reaction time of the 25 beetles tested to all six odors is 15.4 seconds. These insects were confined in a cage in the light near a window. This cage is 30 inches long, 30 inches high and 4½ inches wide. All six sides are wire-screen. A fresh supply of potato plant leaves was constantly kept in the cage. The beetles confined in this cage on the potato plant

leaves appeared "at home" just as much as they do in potato patches. They ate the leaves, copulated and laid eggs as usual. Up to the time of this writing (Jan. 15), 28 of the 45 beetles have died. These lived from 14 to 151 days with 69 days as an average.

(*a*) *Effects with Antennæ Pulled Off.*

Twenty-nine more potato beetles were collected from the potato patch. Their antennæ were pulled off at the bases. A small drop of blood exuded from each wound. These insects were put into the wire-screen cage with the unmutilated ones. Two days later the 23 remaining live ones were tested with only the odors from the three essential oils. All of these beetles were quite inactive and three failed to respond when tested. These three also failed to respond when touched with a pencil. For this reason they were discarded. The general average reaction time of the 20 beetles tested is 3.5 seconds. As a rule these mutilated insects appeared normal in all respects several days after having the antennæ pulled off, because they ate, copulated and were as active as ever. They lived from 2 to 140 days with 38 days as an average.

(*b*) *Effects with Elytra Pulled Off and Wings Cut Off.*

Thirty-one more potato beetles were collected. Their elytra were pulled off at the articulations and the wings were cut off as closely as possible to the articulations. A small drop of reddish or yellowish blood exuded from each wound. The heavy veins, extending from the base of the wing to where the wing folds, contain most of the blood found in these wings. The elytra are also filled with blood. The amount of blood in them gradually diminishes from the base to the distal end. A day after being mutilated 25 of these insects were tested with the six odors. They were apparently normal in all respects except in their slowness in responding to odors. They were as active as unmutilated ones and eight were extremely restless. Their responses were similar to those of unmutilated beetles, except they were not pronounced.

Their reaction times are as follows: Oil of peppermint, 2 to 40

seconds, average 7.8 seconds; oil of thyme, 2 to 15 seconds, average 4.8 seconds; oil of wintergreen, 3 to 60 seconds, average 21.1 seconds. Five failed to respond to this odor. Leaves and stems of pennyroyal, 5 to 60 seconds, average 32.2 seconds. Ten failed to respond to this odor. Leaves and stems of spearmint, 3 to 60 seconds, average 29.8 seconds. Eight failed to respond to this odor. Parts of decayed beetles, 3 to 60 seconds, average 30.4 seconds. Eight failed to respond to this odor. The general average reaction time of the 25 beetles tested to the six odors is 22.7 seconds. In the wire-screen cage with the other potato beetles already tested, these mutilated ones appeared normal, because they ate normally and copulated as much as usual. Since the soft dorsal sides of their abdomens were unprotected, many of them soon began to sink, so that by the time a beetle died, the abdomen had shrunk to about one-fourth its original size. Up to the time of this writing (Jan. 15), 29 of these 31 mutilated insects have died. They lived from 3 to 140 days with 52 days as an average.

(*c*) *Effects with Elytra Pulled Off, Bases of Wings Glued and Pores on Legs Covered with Vaseline.*

Twenty-nine more potato beetles were collected. Their elytra were pulled off at the articulations. Two days later the upper surfaces of the bases of the wings of the 26 remaining live ones were covered with liquid glue. Since the olfactory pores extend a considerable distance from the base of the wing along the radial vein, the glue applied probably did not cover more than 90 per cent. of the pores on each wing. Three hours after applying the glue, the trochanters, femurs and proximal ends of the tibiæ of these beetles were covered with the vaseline-beeswax mixture. An hour still later the insects were tested with the six odors. They were as active as unmutilated ones and appeared normal in all respects except in their responses to odors. Their responses were never pronounced and seldom prompt.

Their reaction times are as follows: Oil of peppermint, 3 to 60 seconds, average 10.7 seconds. One failed to respond to this odor. Oil of thyme, 3 to 60 seconds, average 9 seconds. One failed to respond to this odor. Oil of wintergreen, 5 to 60 seconds,

average 35.9 seconds. Eleven did not respond to this odor. Leaves and stems of pennyroyal, 3 to 60 seconds, average 35.2 seconds. Twelve did not respond to this odor. Leaves and stems of spearmint, 5 to 60 seconds, average 42.6 seconds. Fourteen failed to respond to this odor. Parts of decayed beetles, 5 to 60 seconds, average 40.3 seconds. Fourteen failed to respond to this odor. The general average reaction time of the 26 beetles tested to the six odors is 29 seconds which is twice the reaction time of unmutilated potato beetles to the same odors. When the reaction times to the odors from only the three essential oils are considered, these mutilated insects responded only one fifth as rapidly as did the unmutilated ones. In the wire-screen cage with the other potato beetles already tested, they were apparently normal as long as they lived, because they ate and copulated as usual and were always as active as the unmutilated ones. Before they died their abdomens shrunk considerably in size. Up to the time of this writing (Jan. 15), 28 of the 29 have died. These lived from 2 to 151 days with 61 days as an average.

MELOIDÆ.

The Olfactory Sense of *Epicauta marginata*.

Twenty blister beetles (*Epicauta marginata*), commonly known as the "old-fashioned potato bugs," were caught on clematis. When mechanically irritated, they fold the antennæ and legs against the body, usually eject a small drop of amber-colored liquid from each femoro-tibial articulation, and feign death. On account of this behavior, they were unsatisfactory to experiment with. When put into the experimental cases, some of them lay apparently lifeless for almost a half day. In this state they never respond to any odor, and after becoming as active as usual, they may or may not respond to odors.

When tested with the odors from only the three essential oils, a general average reaction time of 13.9 seconds was obtained. Two of them failed to respond to each of the oils of peppermint and wintergreen. These insects were confined in the cage described on page 436. They were regularly provided with a fresh supply of clematis. In this cage on the clematis they seemed "at home," but they flew out at every opportunity.

They copulated as usual. They lived from 11 to 40 days with 27.6 days as an average.

(*a*) *Effects with Antennæ Cut Off.*

Eight more *Epicauta marginata* were collected. Their antennæ were cut off at the bases. A small drop of amber-colored blood exuded from each wound. Seven days later the two remaining live ones were tested with the odors from the three essential oils. The general average reaction time is 5 seconds. All these beetles were abnormal in behavior. They lived from 1 to 8 days with 3.4 days as an average.

(*b*) *Effects with Antennæ Pulled Off.*

The antennæ of 12 more *Epicauta marginata* were pulled off at their bases. A small drop of blood exuded from each wound. When tested with the odors from the essential oils three days later, the eight remaining live beetles gave a general reaction time of 5.9 seconds. They were less abnormal in behavior than those with the antennæ cut off. They lived from 2 to 13 days with 5.5 days as an average.

(*c*) *Effects with Elytra and Wings Pulled Off.*

The elytra and wings of nine *Epicauta marginata* were pulled off at their articulations. A small drop of blood exuded from each wound. When tested with the odors from the essential oils two days later, the seven remaining live beetles gave a general reaction time of 25.7 seconds. Two of them failed to respond to each of the oils of peppermint and wintergreen. These mutilated insects appeared normal in behavior and in confinement they copulated as usual. They lived from 2 to 14 days with 8 days as an average.

THE OLFACTORY SENSE OF *Epicauta pennsylvanica.*

Twenty-five blister beetles (*Epicauta pennsylvanica*) were caught on golden rod (*Solidago*). This species has the same habit of feigning death when mechanically irritated as has *Epicauta marginata.* When tested with the odors from the essential oils, they gave a general average reaction time of 11.5 seconds

which is only one-half as rapid as the reaction time of the same species devoid of antennæ. Three failed to respond to the oil of peppermint, one to the oil of thyme and two to the oil of wintergreen. A common response was to vibrate the legs. They were placed into the cage with the other species of blister beetles. They were regularly provided with a fresh supply of goldenrod. In the cage they appeared normal, and they copulated as much as usual. They lived from 2 to 25 days with 11.2 days as an average.

(*a*) *Effects with Antennæ Pulled Off.*

The antennæ of 30 *Epicauta pennsylvanica* were pulled off at their bases. When tested with the odors from the essential oils three days later, the 22 remaining live beetles gave a general reaction time of 5.3 seconds. They were only slightly abnormal in behavior. They lived from 2 to 25 days with 8.7 days as an average.

(*b*) *Effects with Elytra and Wings Pulled Off.*

The elytra and wings of 21 *Epicauta pennsylvanica* were pulled off at their articulations. A small drop of blood exuded from each wound. Blood was also seen in the distal ends of the elytra. When tested with the odors from the essential oils two days later, the 17 remaining live beetles gave a general reaction time of nine seconds. One of them failed to respond to the oils of thyme and wintergreen. These insects appeared normal in confinement with the other blister beetles. They copulated as usual. They lived from 1 to 33 days with 10.7 days as an average.

A summary of all the preceding experiments to determine the location of the olfactory organs in beetles is best presented in a tabulated form. The following table is such a summary. Since a comparison of the behavior of unmutilated and mutilated insects alone is not always a safe criterion for judging the general behavior of mutilated beetles, the behavoir of the mutilated beetles recorded in this table is based mostly upon a comparison of the longevities of unmutilated and mutilated individuals of the same species. A "+" after a figure in the last column means that all the insects used in the experiment have not yet died. The longevity is based only on those that have died up to the time of this writing (Jan. 15).

TABLE II.

SUMMARY OF EXPERIMENTS TO DETERMINE THE LOCATION OF THE OLFACTORY ORGANS IN COLEOPTERA.

Species.	Experiment and Behavior of Insects Tested.	Average Reaction Time.		No. of Individuals Tested.	Average Length of Life in Captivity.
		For Three Odors.	For Six Odors.		
		Sec.	Sec.		Days.
Harpalus pennsylvanica.	Unmutilated. Normal in behavior	9.5	16.5	25	61.0+
	Antennæ pulled off. Normal in behavior	5.1	16.1	25	58.0+
	Elytra and wings pulled off. Slightly abnormal in behavior	13.0	17.7	25	9.0
	Elytra and wings pulled off and pores on legs covered with vaseline. Slightly abnormal in behavior	16.9	24.1	18	10.0
Harpalus caliginosus.	Unmutilated. Normal in behavior	4.2		8	Used below
	Antennæ pulled off. Slightly abnormal in behavior	16.4		6	18.0
Epilachna borealis.	Unmutilated. Normal in behavior	13.8		18	Used below
	Antennæ pulled off. Slightly abnormal in behavior	30.8		25	22.0+
	Elytra and wings pulled off. Slightly abnormal in behavior	31.3		4	2.0+
Chaulcognathus pennsylvanica.	Unmutilated. Normal in behavior	2.8		25	3.2
	Antennæ pulled off. Slightly abnormal in behavior	2.8		3	1.3
Passalus cornutus.	Unmutilated. Normal in behavior	3.0	3.2	4	Used below
	Antennæ pulled off. Slightly abnormal in behavior	3.0	3.3	4	12.5
Cotinis nitida	Unmutilated. Normal in behavior	5.0	8.0	1	Used below
	Antennæ pulled off. Normal in behavior	5.6	8.3	1	12.0
Euphoria sepulchralis.	Unmutilated. Normal in behavior	3.6		5	Used below
	Antennæ pulled off. Normal in behavior	4.3		5	20.0
Cyllene robiniæ	Unmutilated. Normal in behavior	5.4		18	10.4
	Antennæ pulled off. Slightly abnormal in behavior	3.0		15	5.0
	Elytra and wings pulled off. Slightly abnormal in behavior	9.8		18	4.2

Species.	Experiment and Behavior of Insects Tested.	Average Reaction Time. For Three Odors.	Average Reaction Time. For Six Odors.	No. of Individuals Tested.	Average Length of Life in Captivity.
		Sec.	Sec.		Days.
Leptinotarsa 10-lineata.	Unmutilated. Normal in behavior	3.8	15.4	25	69.0+
	Antennæ pulled off. Normal in behavior	3.5		20	38.0
	Elytra pulled off and wings cut off. Normal in behavior	11.2	22.7	25	52.0+
	Elytra pulled off, bases of wings glued and pores on legs covered with vaseline. Normal in behavior	18.5	29.0	26	61.0+
Epicauta marginata.	Unmutilated. Normal in behavior	13.9		20	27.6
	Antennæ cut off. Considerably abnormal in behavior	5.0		2	3.4
	Antennæ pulled off. Slightly abnormal in behavior	5.9		8	5.5
	Elytra and wings pulled off. Slightly abnormal in behavior	25.7		7	8.0
Epicauta pennsylvanica.	Unmutilated. Normal in behavior	11.5		25	11.2
	Antennæ pulled off. Slightly abnormal in behavior	5.3		22	8.7
	Elytra and wings pulled off. Normal in behavior	9.0		17	10.7

A summary of the preceding table shows the following: After the antennæ were pulled off, four of the 11 species tested were normal and seven were slightly abnormal in behavior. After the elytra and wings were pulled off one species was normal while four were slightly abnormal in behavior. After the elytra were pulled off and the wings were cut off, the one species tested was normal in behavior. After the elytra and wings were pulled off and the pores on the legs were covered with vaseline, the one species tested was slightly abnormal in behavior. After the elytra were pulled off, the bases of the wings glued and the pores on the legs covered with vaseline, the one species tested was normal in behavior.

Four unmutilated species responded to odors more slowly than did the same species after the antennæ had been pulled off. This is explained by the fact that most beetles are more or less "timid"

for some time after being caught, and some feign death. As a rule the longer they are confined and the more they are handled, the more satisfactory they are to experiment with. Five species without antennæ responded to odors as promptly as did the same species unmutilated. Two species without antennæ responded to odors more slowly than did the same species unmutilated. Since these were abnormal in behavior and judging from the reaction times of the other nine species with antennæ pulled off, it is only reasonably to attribute the slow reaction times of these two species to their abnormal condition caused by the antennæ being pulled off. The six species so mutilated that most of their olfactory pores on the elytra and wings were prevented from functioning responded from two to five times more slowly than did the same species unmutilated or with the antennæ pulled off. The two species so mutilated that most of their olfactory pores on the elytra, wings and legs were prevented from functioning responded from two to six times more slowly than did the same species unmutilated or with the antennæ pulled off.

From all the preceding results, it seems that the antennæ do not carry any of the olfactory organs, while the olfactory pores found on the peduncles of the elytra, on the dorsal surfaces of the wings, on the trochanters, tibiæ, sometimes on the femurs and tarsi, and perhaps on the mouth appendages, are the true olfactory organs in beetles.

SUMMARY.

In making a comparative study of the olfactory pores in beetles, 50 species belonging to 47 genera and representing 34 families were used. A group of pores is always present on the peduncle of each elytron. It lies on the dorsal side of the well-exposed radial plate. The number of pores on a pair of elytra varies from 12 to 310. As a rule, the more pores in the group the smaller they are and the closer they are together.

Of the 47 winged species examined, 11 have only one group of pores on each wing, 21 have two groups on each wing, 12 have three groups on each wing, and 3 have four groups on each wing. These groups are always located on the dorsal surface. Only occasionally are a few scattered pores found on the ventral side

of a wing. When one or two groups are present, they lie on the radius. When three groups are present, all three may lie on the radius, or two may lie on the radius and the third on the media. When four groups are present, one lies on the subcosta, two on the radius and one on the media. The largest group on the radius usually extends nearly all the way to the fold of the wing and sometimes all the distance to the fold. The number of pores on a pair of wings varies from 130 to 982.

There are usually two groups of pores at the proximal end of each trochanter. Sometimes a pore is found at the proximal end of the femur. It is common to find a few pores at the proximal end of each tibia; and sometimes pores are found in the tibial spines and on the tarsi. The number of pores on all six legs varies from 49 to 341.

In regard to water beetles, the better the legs are adapted for locomotion in water, the fewer pores they have. The smallest winged species (*Coxelus*) examined has 273 pores, which is the smallest number of all the species, and the largest species (*Orthosoma*) has 1,268 pores which is the largest number of all the species examined. The apterous species have more pores on the legs than usual. As a rule, the smaller the species, the fewer its pores and the larger they are, comparatively speaking. As a rule, there are no generic and specific differences, except variations in number of pores, the amount of variation depending on the sizes of the individuals compared. There are no individual and sexual differences other than slight variations in number of pores.

The pore apertures or pits are round, oblong, slitlike or club-shaped. On the elytra and wings they are always round or oblong. On the legs they have all four of the enumerated shapes.

The spindle-shaped sense cells of most beetles lie in the lumens of the appendages outside the pore cavities, but in the legs of *Orthosoma* the sense cells lie inside the pore cavities. A small chitinous cone is always present. It is formed by the hypodermal cell at the mouth of the pore after the insect has emerged from the last pupal stage, and at the same time when the chitinous integument is being considerably thickened. The sense cells are fully developed when the insect emerges into the imago stage. The sense fiber pierces the cone and the layer of chitin between

the pore aperture and cone, and it enters the bottom of the pore aperture or pit where its peripheral end comes into direct contact with the outside air. In Hymenoptera the sense fibers enter the pore apertures which are almost on a level with the external surface of the chitin. In Coleoptera, with a few exceptions, the sense fibers enter the bottoms of pits which lie in the chitin one third (at time of emerging into imago stage) the distance from the external surface. In the legs of the lady beetle, *Epilachna borealis*, instead of the chitin which surrounds the pore apertures being depressed, it is elevated so that the pore apertures lie in the center of domes above the general surface of the legs. In the legs of the blister beetles, *Epicauta marginata* and *E. pennsylvanica*, the pore apertures lie on a level with the surface of the legs. In the legs of the potato beetle, the pore apertures lie at the bottoms of shallow pits. All four preceding species have hypodermal gland pores over the entire body, except the wings. These pores in the lady beetle are perhaps the most highly developed. They lie on all sides and even among the olfactory pores on the legs. In the other three species they are less highly developed on the legs near the olfactory pores and none is found very close to an olfactory pore. This correlation between the hypodermal gland pores and the olfactory pores is certainly a means of preventing the secretion from the gland cells from running into the pore apertures.

A large nerve and a large trachea run into each elytron and wing. In the peduncle of the elytron they run through the radial plate just beneath the group of olfactory pores. Branches from the nerve are given off which connect with the sense cells. The large nerve and trachea passing into the wing soon divide so that a smaller nerve and a smaller trachea run through each main nerve. The largest trachea passes through the subcosta, and the largest nerves pass through the veins carrying the olfactory pores. These nerves give off branches which connect with the sense cells. The sense cells wherever found are always surrounded by blood.

In the experiments to determine the location of the olfactory organs, 434 individuals were tested. These belonged to 11 species representing 8 families. After the antennæ were pulled

off, 4 of the 11 species tested were normal and 7 were slightly abnormal in behavior. After the elytra and wings were pulled off 1 species was normal while 4 were slightly abnormal in behavior. After the elytra were pulled off and the wings were cut off, the 1 species tested was normal in behavior. After the elytra and wings were pulled off and the pores on the legs were covered with vaseline, the 1 species tested was slightly abnormal in behavior. After the elytra were pulled off, the bases of the wings glued and the pores on the legs covered with vaseline, the 1 species tested was normal in behavior.

Four unmutilated species responded to odors more slowly than did the same species after the antennæ had been pulled off. This is explained by the fact that most beetles are more or less "timid" for some time after being caught, and some feign death. As a rule, the longer they are confined and the more they are handled, the more satisfactory they are to experiment with. Five species without antennæ responded to odors as promptly as did the same species unmutilated. Two species without antennæ responded to odors more slowly than did the same species unmutilated. Since these were abnormal in behavior and judging from the reaction times of the other 9 species with antennæ pulled off, it is only reasonable to attribute the slow reaction times of these two species to their abnormal condition caused by the antennæ being pulled off. The 6 species so mutilated that most of their olfactory pores on the elytra and wings were prevented from functioning responded from 2 to 5 times more slowly than did the same species unmutilated or with the antennæ pulled off. The two species so mutilated that most of their olfactory pores on the elytra, wings and legs were prevented from functioning responded from 2 to 6 times more slowly than did the same species unmutilated or with the antennæ pulled off.

From all the preceding results, it seems that the antennæ do not carry any of the olfactory organs, while the olfactory pores found on the peduncles of the elytra, on the dorsal surfaces of the wings, on the trochanters, tibiæ, sometimes on the femurs and tarsi, and perhaps on the mouth appendages, are the true olfactory organs in beetles.

DISCUSSION.

Since the writer ('14*c*) has already written a complete review of all the literature available concerning the sense of smell in insects, only a brief discussion is necessary in this paper.

Hicks ('57) says that the olfactory pores in Coleoptera are arranged in long rows along the subcostal nerves. The same author ('59) states that in Coleoptera these organs are highly developed and occur in numerous groups on the subcostal vein, mostly at the widest part, but are also scattered along it to the fold of the wing. In *Carabus* they are found on veins other than the subcostal. In many beetles the pore is overarched by a hair, which probably protects the organ. He could distinguish no sexual differences in these organs, except the pores are slightly larger in the females, due to their greater size. Hicks ('60) first found the olfactory pores on the legs of beetles. The present writer has never seen a hair overarching an olfactory pore.

Hochreuther ('12) seems to be the first to study the internal anatomy of the olfactory pores in beetles. Since he used only *Dytiscus marginalis* and perhaps because he did not have enough sections through these organs, he failed to understand their anatomy. He states that each dome-shaped organ is located at the bottom of a chitinous flask, the mouth of which communicates with the exterior. Instead of the peripheral end of the sense fiber coming into direct contact with the air in the flask, it apparently stops just beneath the chitinous dome at the top of the organ. His terminal strand (Terminalstrand) may be the same as the hypodermal secretion forming the cone described by the writer. Hochreuther found a few of these dome-shaped organs on the epicranium near the margin of the eyes, nine on the proximal end of the first antennal segment, two on the distal end of the second antennal segment, a few on the dorsal side of the labrum, a very few on the dorsal side of the mandible, several on each maxilla, about 18 on the first four segments of the front legs, about 10 on the first three segments of the middle legs, and a few on the trochanters of the hind legs. He evidently did not examine the wings. Thus according to Hochreuther these organs are rather widely distributed. Since the peripheral ends of the sense fibers do not come into contact with the outside air,

but connect with the tops of the domes, he suggests that they receive some kind of mechanical stimuli, although he performed no experiments to determine their function.

Lehr ('14), resuming the search for sense organs in *Dytiscus marginalis* where left off by Hochreuther, found dome-shaped organs on the elytra and wings. He found three main groups in identically the same places as described by the present writer. The number of pores in the group on the elytron varies from 130 to 150. The two main groups on the radius (his subcosta) of the wing are large, but he did not count the pores in them. He found a fourth group, consisting of about 30 pores, on the ventral side of the costa near the base of the wing. He also found a few scattered pores on the dorsal side of the costa just distal to the fold of the wing, a few on the second cubitus, and a few irregularly scattered along the full length of the media. Lehr has described the anatomy of these organs almost identically as seen by the present writer, but it seems that he has not correctly interpreted some of the structures. He seems to think that each sense cell is surrounded by another cell, but the latter cell is perhaps nothing more than coagulated blood and the portion of it extending into the pore is certainly a hypodermal secretion forming the cone as described in the preceding pages. His neurilemma nuclei are perhaps hypodermal nuclei. He is able to trace the sense fiber through the cone, but he has not recognized the small opening through the dome. This is not surprising, because the pores in the wings as so small that the openings or pore apertures are never noticed unless first seen in the largest pores in the legs or mouth parts. In the thinnest sections, the chitin forming the dome is so thick as compared to the diameter of the pore aperture that the aperture appears only as a streak slightly lighter than the other chitin in the dome. Lehr has nothing to say about the physiology of these organs.

In experimenting with mutilated beetles, Hauser ('80) seems to be the only one who has taken their longevity into consideration. And even he has not kept an accurate record of their behavior and longevity. He claims to have studied the behavior of beetles before and after the removal of the antennæ. When the antennæ were removed he ascertained that many beetles

soon became sick and died, while others lived thereafter for many days. When tested with odors, most of the beetles without antennæ failed to respond, but Hauser states that *Carabus*, *Melolontha* and *Silpha* still responded to odors, although more slowly.

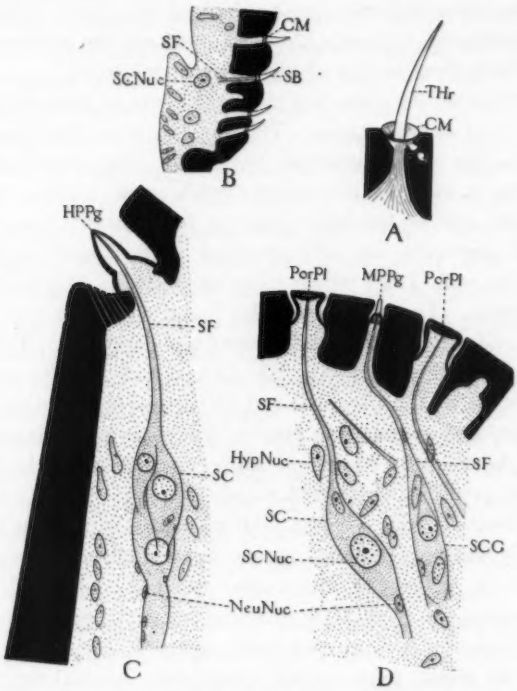

FIG. 3. Antennal organs of the water beetle, *Dytiscus marginalis*, copied from Hochreuther (1912). *A*, small tactile hair (Sinneshaar) from first segment of antenna, total preparation (Fig. 1 from Hochreuther), × 330; *B*, portion of Fig. 12 from Hochreuther, showing four small sense bristles (Sinnesborsten) from proximal end of second segment of antenna, × 265; *C*, longitudinal section (Fig. 48 from Hochreuther) through a hollow pit peg (hohlen Grubenkegel), × 470; *D*, longitudinal section through a small massive pit peg (massiven, grubenständigen Zapfen) and two pore plates (kelchförmige Organe), × 590. This drawing is a combination of Figs. 32 and 58 from Hochreuther. Only the pore plates (*PorPl*) are taken from Fig. 58. Hochreuther gives a drawing of only one perfect pore-plate organ, or cup-shaped organ, and it is from the maxillary palpus. *CM*, cup-shaped membrane; *HPPg*, hollow pit peg; *MPPg*, massive pit peg; *PorPl*, pore plate; *SB*, sense bristle; *THr*, tactile hair. See page 456 for other abbreviations.

For the purpose of judging whether the antennal organs are better adapted anatomically than the olfactory pores for receiving odor stimuli, the former organs (Text-fig. 3, p. 453), of *Dytiscus marginalis* have been copied from Hochreuther ('12). This work of Hochreuther is a comprehensive study of the morphology of all the chitinous sense organs of *Dytiscus*. Since it is perhaps the latest and certainly the best study on the antennal organs of beetles, these organs shall be briefly described.

Each of the 11 segments in the antenna of *Dytiscus* carries a number of sense organs. The farther from the base of the antenna the more numerous they are. The distal half of the antenna is covered abundantly with sense organs, while the proximal half is sparingly covered with them. The first and second segments are well provided with slender tactile hairs (Text-fig. 3*A*, *THr*) which have been called *Sensilla trichodea* by Schenk. These hairs are also found on all the other appendages and even on the head, thorax and abdomen. Two groups of sense bristles (Text-fig. 3*B*, *SB*), called *Sensilla chætica* by Schenk, lie at the proximal end of the second segment. These hairs are also common on most of the other appendages, on the head, thorax and abdomen. All segments, except the first one, are well provided with small massive pit pegs of the thick-walled type (Text-fig. 3*D*, *MPPg*). All segments, except the first one, are only sparingly provided with a second type of pit pegs. This one is the hollow or thin-walled type (Text-fig. 3*C*, *HPPg*). Only about six of these were found on each segment. Besides being found on the antennæ, both types of pit pegs are common on all the mouth parts, on the mesothorax, around the spiracles, on all the legs, and on the sexual apparatus. Pit pegs have been called *Sensilla coelloconica* by Schenk. All segments, except the first two, are abundantly supplied with the cup-shaped or pore-plate organs (Text-fig. 3*D*, *PorPl*). For both antennæ they are estimated between 4,500 and 5,000. These organs are also common on the palpus of the first maxilla. They were first studied by Nagel on the antennæ and maxillary palpi of Dytiscidæ. In the honey bee Schenk has called them *Sensilla placodea*. Of the five antennal organs of *Dytiscus*, only the hollow pit pegs are regarded by Hochreuther as probably ol-

factory in function. If they really act as olfactory organs, then the mouth parts, thorax, legs and sexual organs must aid in receiving odor stimuli. Hochreuther considers the antennæ more important as appendages for carrying organs for receiving mechanical stimuli rather than those receiving chemical stimuli.

According to various authors the antennal organs of different beetles vary only slightly. The antennal organs of *Dytiscus* are also similar to those of the honey bee. In both of these insects the tactile hairs are of the same type. The Forel flasks and pit pegs of the honey bee are two types of pit pegs which are perhaps rudimentary, because the tips of the hairs do not come to the exterior of the chitin. The massive pit pegs, hollow pit pegs, and the sense bristles of *Dytiscus* are certainly nothing more than three types of tactile hairs. The hollow pit pegs compare closely with the pegs of the honey bee, except the pegs have thinner chitin at the tips. This is probably on account of more acute sense of touch in the honey bee. The pore-plate organs of the honey bee and the cup-shaped organs of *Dytiscus* are also quite similar.

One or more of the antennal organs of every insect studied have been called olfactory organs, and it is possible that most of these organs may be found on other appendages, besides the antennæ, as already seen in *Dytiscus*.

In conclusion it seems beyond a doubt that none of the antennal organs of beetles shown in Text-fig. 3 serves as an olfactory organ, and that the olfactory pores are well adapted anatomically for receiving odor stimuli, because the peripheral ends of their sense fibers come into direct contact with the external air.

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EXPLANATION OF PLATES I. AND II.

All figures including Text-figs. 1 and 2 are from camera lucida drawings made at the base of the microscope. Figures 1 to 8 inclusive and 22 on the plates are enlarged 465 diameters. All the remaining figures on the plates, except the diagrams 28, 29 and 31, are enlarged 580 diameters.

Abbreviations.

BlSin.....blood sinus.
BM.....basal margin of elytron.
C.....costa.
Ch.....chitin.
Ch$_1$.....chitin formed before insect emerges into imago stage.
Ch$_2$.....chitin formed after insect emerges from last pupal stage.
ChM.....chitinous membrane of pore plate.
CM.....cup-shaped membrane of tactile hair on antenna.
Con.....chitinous cone.
ConT.....connective tissue.
ConTNuc....nucleus of connective tissue.
Cx.....coxa.
F.....femur.
Fl.....flange of olfactory pore.
Fo.....where wing folds.
GlC.....gland cell.
HPPg.....hollow pit peg on antenna.
Hr.....hair.
Hyp.....hypodermis.
Hyp$_1$.....membrane resembling hypodermis which divides the lumen of proximal end of the tibia of *Epilachna* into two chambers.
HypC.....hypodermal cell.
HypNuc.....hypodermal nucleus.
HypS.....hypodermal secretion.
M.....muscle.
MD.....muscle disk.
Me.....media.
Mo.....mouth of pore.

MPPg.....small massive pit peg on antenna.
N.....nerve.
NB.....nerve branch.
Neu.....neurilemma.
NeuNuc.....nucleus of neurilemma.
NeurNuc.....neuroglia nucleus.
P.....pit of pore.
PorAp.....pore aperture.
PorGl.....pore of gland.
PorHr.....pore of hair.
PorPl.....pore plate on antenna.
Por.....pore of olfactory organ.
PorW.....pore wall.
PorWGl.....pore wall of gland.
PorWHr.....pore wall of hair.
R.....radius.
RP.....radial plate.
SB.....small sense bristle on antenna.
SC.....sense cell.
SC_1.....sense cell of tactile hair
SCG.....sense cell group.
SCNuc.....sense cell nucleus.
SCNucl.....sense cell nucleolus.
Sc.....subcosta.
ScH.....subcostal head.
SF.....sense fiber.
Tar.....tarsus.
Tb.....tibia.
TbSp.....tibial spine.
THr.....tactile hair.
Tr.....trachea.
TrNuc.....Nucleus of trachea.
Tro.....trochanter.
1 to 6.....groups Nos. 1 to 6 of the olfactory pores.
a.....location of scattered pores on ventral side of wing.
b and *c*.....location of scattered pores on tibia.

PLATE I.

FIG. 1. Six of the eight olfactory pores (*PorW*) in group 6 on inner surface of right hind leg of *Epilachna borealis;* also one hair (*Hr*), one hair socket (*porWHr*) and two hypodermal gland pores (*PorWGl*).

FIG. 2. Two olfactory pores (*PorAp*), five hairs (*PorWHr*) and 19 gland pores (*PorWGl*) on outer surface at proximal end of right hind leg of *Epilachna*.

FIG. 3. Five olfactory pores from tibial spine of *Epicauta marginata* (same as shown in Text-fig. 2*G*).

FIG. 4. Group 1 of olfactory pores on peduncle of elytron of *Epilachna* (same as shown in Text-fig. 1*B*).

FIG. 5. Seven of the olfactory pores in group 2 on wing of *Epilachna*.

FIG. 6. Eleven of the olfactory pores in group 3 on wing of *Epilachna*.

FIG. 7. Ten of the olfactory pores in group 4 on wing of *Epilachna*.

FIG. 8. Four of the five olfactory pores on ventral side of wing of *Epilachna*. Figs. 5 to 8 represent some of the pores as shown in Text-Fig. 1*C*.

FIG. 9. Olfactory pore from trochanter of *Uloma*.

FIG. 10. Olfactory pore from tibia of *Uloma*.

FIG. 11. Three olfactory pores from elytron of *Uloma*.

FIG. 12. Olfactory pore and sense cell from wing of *Uloma*.

FIG. 13. Olfactory pore and sense cell from trochanter of *Orthosoma* (cut slightly obliquely).

FIG. 14. Olfactory pore and about one third of sense cell (*SC*) from trochanter of *Orthosoma*, showing pit (*P*) and flange (*Fl*).

FIG. 15. Olfactory pore from tibia of *Orthosoma*.

FIG. 16. Oblique section through trochanter of *Epilachna*, showing anatomy of leg. It was cut in such a manner that no muscles are shown in the section and that the nerve (*N*) is severed in two places.

FIG. 17. Cross section through proximal end of tibia of *Epilachna*, showing anatomy of leg at this place. The gland pore (*PorGl*), hair pore (*PorHr*) and sense cells (*SC*), belonging to the tactile hairs (*THr*) were taken from two other sections, and the gland cell just beneath the gland pore was taken from the other end of this section.

FIG. 18. Four olfactory pores and a small portion of hypodermis from elytron of *Epilachna*. The material used for Figs. 17 and 18 was from an old adult beetle that had been confined in the laboratory nearly all summer.

FIG. 19. Four olfactory pores, sense cells and nerve (*N*) from wing of *Epilachna*.

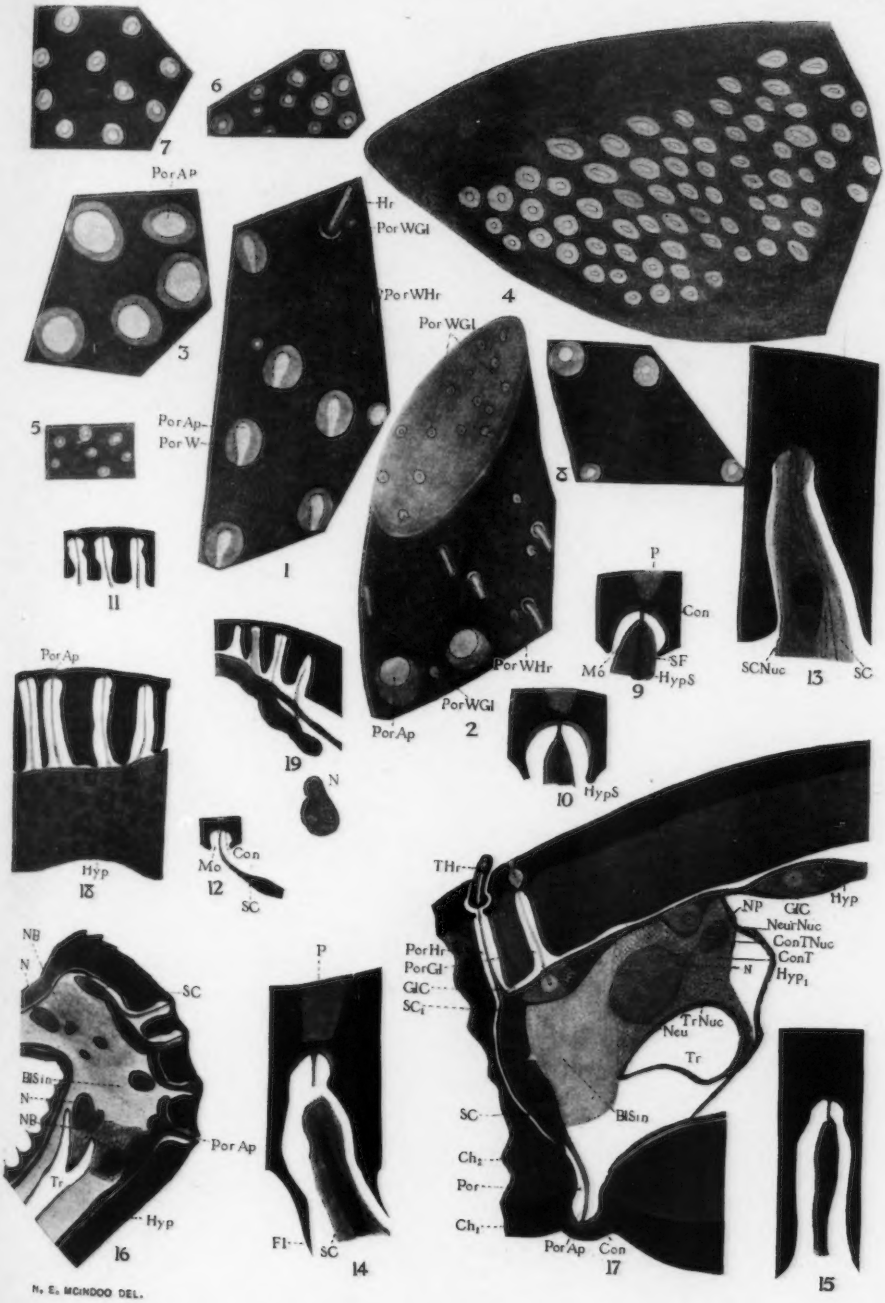

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PLATE II.

FIG. 20. Three olfactory pores from wing of *Passalus*.

FIG. 21. Six olfactory pores from elytron of *Orthosoma*.

FIG. 22. Cross section through wing of *Orthosoma*, showing anatomy of wing beneath olfactory pores.

FIG. 23. Olfactory pore from elytron of *Passalus*, showing sense cell (*SC*) connected with pore aperture (*PorAp*) and with nerve (*N*); also hypodermal cell (*HypC*) that forms the cone (*Con*).

FIG. 24. Olfactory pore from trochanter of *Epicauta marginata*.

FIG. 25. Olfactory pore from trochanter of *Epicauta pennsylvanica*.

FIG. 26. Olfactory pore from trochanter of *Leptinotarsa 10-lineata*.

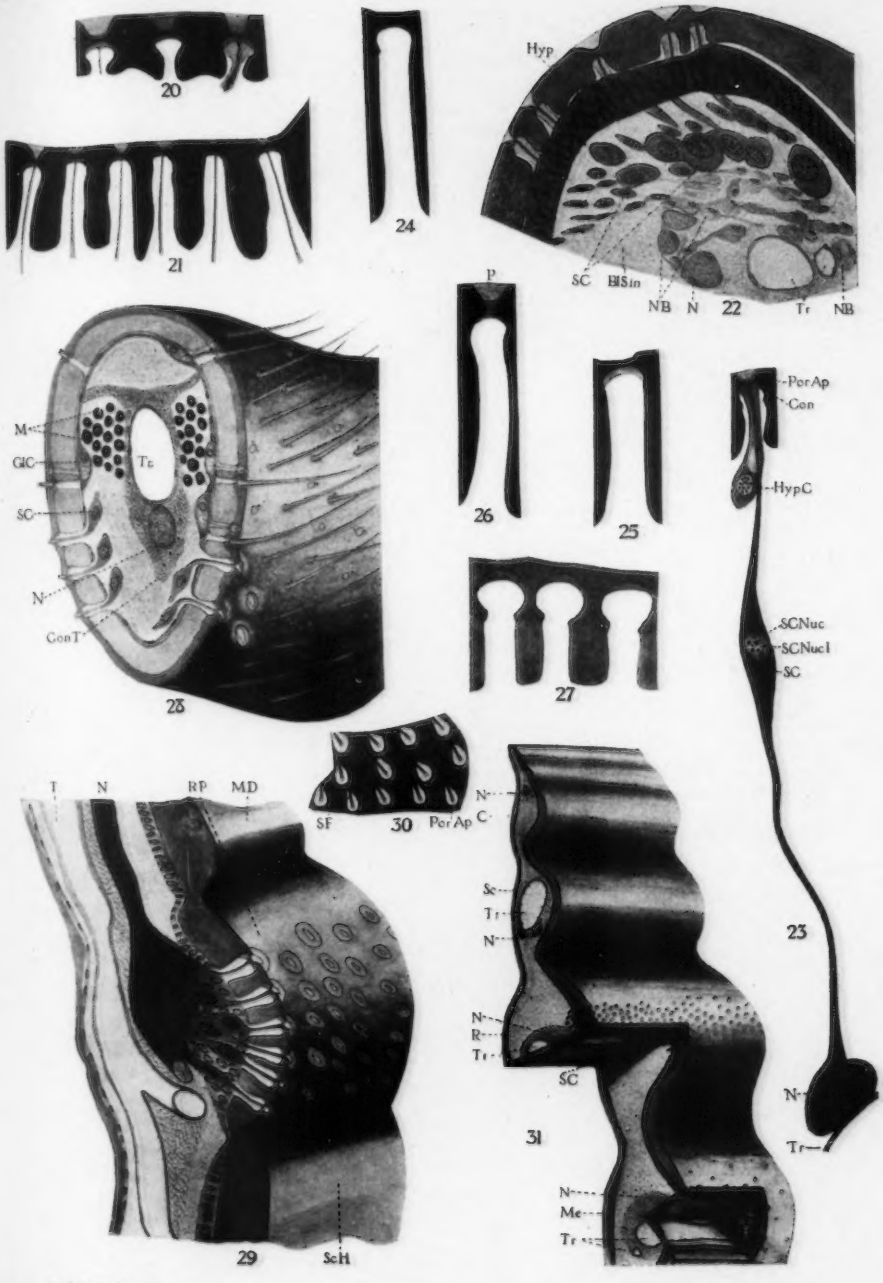
FIG. 27. Three olfactory pores from tibial spine of *Epicauta marginata*. The material used for Figs. 24 to 27 had been treated with caustic potash.

FIG. 28. Transverse-longitudinal diagram of proximal end of trochanter belonging to right hind leg of *Epilachna*, showing internal anatomy of leg and superficial view of hairs, hair sockets, gland pores and olfactory pores. The four pores at the right belong to group 6 and the three at the left belong to group 5.

FIG. 29. Oblique transverse-longitudinal diagram of portion of peduncle belonging to *Epilachna*, showing internal anatomy of radial plate (*RP*), innervation of olfactory pores and a superficial view of a few of the pores in group 1. The transverse portion of the diagram passes through the radial plate in the direction of the line marked "a" in text Fig. 1*B*.

FIG. 30. Oblique superficial view of olfactory pores on wing of *Epilachna*, showing sense fibers (*SF*) connected with pore apertures (*PorAp*).

FIG. 31. Transverse-longitudinal diagram of portion of wing belonging to *Orthosoma*, showing internal anatomy of wing, innervation of olfactory pores and a superficial view of a few of the pores on radius (*R*) and media (*Me*).



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